CLINICAL AND EXPERIMENTAL STUDIES OF SIMPLE AND MALIGNANT GOITRE

BY

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CONTENTS

VOLUME I

PREFACE

The work for this thesis was carried out during the past six years, while the author was a research fellow and then lecturer in pharmacology and therapeutics in the University of Sheffield. It was stimulated by the observation that thyroid enlargement was seen more frequently in Sheffield than in Edinburgh, where the author had trained and worked previously, and is conveniently divided into four parts. The first deals with epidemiological investigations of simple goitre and results of clinical studies on simple goitre in Sheffield. It is followed by studies on the occurrence of goitrogenic substances in milk, the effect of season on such material, and aspects of iodine metabolism in thyroid enlargement produced by goitrogens. The third part describes some aspects of the pharmacology and metabolism of antithyroid substances in relation to their importance in simple goitre. Finally malignant disease of the thyroid is considered with special reference to its development as a complication of simple goitre.

A conventional plan has not been strictly followed in that the results of other workers are quoted for comparison purposes when the results of the present work are described, and not kept for the discussion on each chapter. This is convenient as

there is a vast amount of previous work on this subject. The literature has not been exhaustively reviewed, but a critical examination of previous relevant work has been attempted, especially studies on simple goitre in Britain. A synthesis of the clinical and experimental results is given at the end of the thesis.

INTRODUCTION

In 1838 James Inglis, a graduate of the University of Edinburgh, wrote a book entitled "Treatise on English Bronchocele with a few remarks on the use of iodine and its compounds." (161). He had come from Edinburgh to be physician to the Ripon Public Dispensary in the West Riding of Yorkshire, and was immediately impressed by the high incidence of benign enlargement of the thyroid gland in Ripon and its environs, compared to Edinburgh. He stated in his preface "When I first entered on my duties at Ripon, as Physician to the Public Dispensary, I was surprised to find so many goitrous cases on the records of that Institution; further enquiry however, and personal observation persuaded me that this disease is as much endemic in some of the districts of Yorkshire, and other English Counties, as in Geneva or any of the Alpine valleys." Later he concluded "in the lower parts of Edinburgh, where filth and misery are combined, the disease is almost unknown. In the neighbourhood of Ripon, where there are no humid valleys, nor any extent of poverty and its consequences, we have Goitre to a very great degree; we see it in the upper ranks of society, and we find it in the lower."

Almost one hundred and twenty years later, the author came to Sheffield from Edinburgh and was impressed by a similar geographical difference in

simple goitre to that noted by Inglis. In the early 19th century transport was difficult, communities were isolated and inbreeding was striking. With the passing of a century these differences have disappeared, standards of living are more uniform, so that geographical differences in goitre incidence are even more striking. Corroboration of these regional variations in goitre incidence has appeared in numerous reports during the past hundred years. High incidences of goitre were noted in Cornwall, Somerset, Dorset and Wiltshire in the South of England, and this high incidence extended northwards into Oxfordshire, Warwickshire, Northamptonshire, Derbyshire, Yorkshire and Durham. These findings were given in reports during the 19th century (20,40,165,197, 198 226,293, 299). The incidence of goitre in Scotland has always been reported to be low, but some goitre was found in the Southern Uplands during the 19th century (248). During this century a similar regional incidence of simple goitre was noted in England by Berry (33). In 1924 the Board of Education (33) organised a survey of goitre in England and found striking regional variations, similar in distribution to previous reports. Then in 1931, 1936 and 1944 the Medical Research Council conducted goitre surveys in certain counties of England and Scotland (262,395, 256), and again very large

regional differences in goitre incidence were found.

The evidence that the aetiology of simple goitre in Great Britain is solely due to deficiency of iodine has never been so convincing as the reports from other countries,; particularly of Marine and co-workers from the United States (232,233,234,235); of Wespi (379) Schaub (301), Bayard (29), and Messerli (243) from Switzerland; of Hercus and co-workers from New Zealand (146,147,148,149); and more recently Stanbury et al.(324) from Mendoza. In 1931, Turton (346) fully investigated simple goitre in Derbyshire, and was unable to correlate the regional variations within the county with iodine contents of soil or water. The investigations by the Medical Research Council in 1936 and 1944 demonstrated rough correlations of goitre incidence and iodine contents of water and pasturage, but many exceptions were noted (395, 256). Thus the goitre incidence in Somerset was found to be almost twenty times as great as that in Suffolk, and though the iodine contents of water and pasturage were lower in Somerset, no difference was found in iodine contents of milk from both regions. Similarly high incidence of goitre was noted with both low and high iodine contents of water and the reverse also applied. Thus the high incidence of goitre (48%) of adult women in the Forest of Dean was associated with an iodine content of water, which was higher than all

but 3 of 64 samples of water from various parts of Scotland, where goitre incidence was low. Factors other than iodine have been suggested by these reports, in particular an increased calcium intake from hard water. Recently Koutras et al. (181) have investigated iodine metabolism in patients with simple goitre in Glasgow, and noted lower intakes and urinary excretions of iodine compared to controls. It is noteworthy, that other observers from other countries have found similar differences, but often the intakes and excretion of the control groups have been lower than those with simple goitre in other countries. Thus Hercus and Roberts (149) found a lower intake and excretion of iodine in their control group than Koutras et al.(181) in their patients with simple goitre. Similarly Virtanen and Virtanen (359) in Finland found lower iodine excretion in non-goitrous adults than either of the goitrous groups from Glasgow or New Zealand. This strongly suggests that deficiency of iodine partly accounts for the development of simple goitre, but that other factors must also be implicated, and that demonstrations of lower intakes and excretions of iodine do not prove that deficiency of iodine is the sole cause of the thyroid enlargement.

The theme of the present work is the demonstration of the occurrence and importance of other possible

factors in the development of simple goitre, and in particular the ingestion of goitrogenic substances. The importance of naturally occurring goitrogens has been suggested for some years by the work of Astwood et al. (18), and Greer (125,128, 129), and direct evidence has recently been given by the investigations of Clements and co-workers (70, 71, 116), who have shown the presence of goitrogenic activity in milk in Tasmania. In the present work the following lines of inquiry have been pursued. Most of the previous surveys on goitre incidence have been carried out by examination of school children, and regional variations in goitre incidence in adults have been rarely investigated. Accordingly an attempt to find out the incidence of thyroid enlargement in the general population has been carried out in two regions, Sheffield and Ormiston, a small town in East Lothian, as it was thought that differences in goitre incidence would be present. A further opportunity to study goitre incidence in school-children and the effect of supplementary iodine on thyroid enlargement in Oxfordshire and Wiltshire was carried out with the author present as a member of a joint investigation team from the Ministries of Health and Education, and the Medical Research Council. A large number of patients attending hospital in Sheffield because of simple thyroid enlargement have been investigated to elucidate iodine metabolism in

simple goitre. Uptake and discharge of radioiodine, total content of iodine in the thyroid, rate of turnover of iodine and blood thyroid hormone level have been measured in patients with simple goitre and compared with findings in patients without thyroid enlargement. In addition renal clearance of iodide has been measured and the rate of organic binding of iodine in simple goitre has been assessed. These findings are interpreted in relation to the known disturbances of iodine metabolism produced by iodine deficiency and presence of goitrogens. Direct evidence regarding the occurrence of goitrogens has been obtained by examining milk for antithyroid activity both in man and in rats. Acute and chronic administration has been studied in rats using fresh and dried milk, and seasonal variations investigated. The effects of other seasonal influences have been studied, and also seasonal variations in thyroid size in animals. One of the greatest difficulties about accepting antithyroid substances as a cause of simple goitre in man has been the almost constant demonstration of high uptakes of radioiodine in simple goitre, whereas the usual methods of testing the activity of antithyroid substances have shown depression of uptakes. As only small amounts of goitrogens are likely to be ingested, the effects produced by chronic administration of small quantities of a particular

antithyroid substance, carbimazole, have been studied in rats. Perhaps the most difficult point in the assessment of the importance of goitrogens in human goitre is that the effects of such goitrogens when taken in small amounts over long periods must be surmised from the effect of goitrogens given acutely on the radioiodine uptake of the thyroid gland. In an attempt to study this correlation, acute and chronic administration of a large number of antithyroid compounds with the same basic structure has been carried out, and the effect of small changes in chemical structure of these compounds studied. The metabolism of antithyroid compounds has been little studied, and is of obvious importance in relation to goitrogenic action. Two antithyroid compounds have been labelled with radiosulphate and their metabolism and excretion measured. As the duration of action of these compounds is dependent on their rate of destruction, it is important to elucidate the processes concerned and possible inhibitors. This aspect of metabolism of antithyroid compounds has also been studied.

Finally, consideration has been given to the most important consequence of simple goitre that has been postulated, which is the increased incidence of thyroid carcinoma. A large number of patients with malignant disease of the thyroid have been studied,

with reference to aetiological factors such as previous hyperplasia, due to simple goitre or hyperthyroidism and an attempt to correlate these with the biological characteristics of thyroid tumours has been made. The importance of previous therapy with ionising radiation in the production of thyroid tumours is developed in this study, and is followed by experimental results on the effect of small doses of ionising radiation on rat thyroid function.

Many terms are applied to benign enlargement of the thyroid. De Smet (88) and many other authorities state that endemic goitre exists when more than 10% of the population show clinical signs of generalized or localized thyroid hypertrophy, and that sporadic goitre should be applied to regions where the incidence is less. This is an arbitrary division and ignores the different criteria used in defining thyroid enlargement, which may place a region in one or other category, depending on the standards used. In the present work the term simple goitre has been preferred, which makes no assumptions about incidence, and covers both diffuse and nodular thyroid enlargement. It is considered distinct from specific thyroid enlargement due to hyperthyroidism, lymphocytic or other forms of thyroiditis, and malignant disease.

PART I

CLINICAL STUDIES OF SIMPLE GOITRE

1.

METHODS

Clinical

Assessment of size of thyroid gland. Many observers only note easily visible thyroid glands when performing goitre surveys, and the study group on endemic goitre of the World Health Organisation supported this in 1952 (332). Their classification of size of gland into three stages is therefore necessarily coarse, as stage 1 is an easily visible gland. Murray et al. (256) in England and Perez et al. (268) in Central America have emphasised the importance of estimating the frequency of milder degrees of enlargement which are not necessarily visible and may only be noted by palpation. Visibility is dependent on the type of neck, and may be obscured in a very muscular or fat neck. It is generally accepted that the normal thyroid gland weighs 15-25 g (227, 206, 8, 88), though it is smaller than this in iodine-rich areas such as Iceland (311). Any gland greater than 25 g. may be assumed to be enlarged.

The method of examination used was to inspect the neck from the front, and the patient was then asked to swallow. A decision was then made whether the thyroid gland was visible. Visible swellings were confirmed to be thyroid in origin by palpation, which was performed from behind the patient. An

estimate was made of thyroid size from this palpation, and asymmetry, consistency, and presence or absence of nodules noted. The classification used is shown in Figs. 1 & 2, which show the cards which were used in thyroid gland surveys in Sheffield and Ormiston. It was found from observations on patients in hospital, and follow-up at operation or autopsy, that a gland which was more than 40 g. was almost invariably visible. The visibility of glands which were enlarged but less than 40 g. in size, was dependent on type of neck as well as size of gland. Thus a gland of 30 g. might be visible in a thin neck, and one of nearly 40 g. not visible in a very fat neck. Nevertheless a gland which is under 40 g. in size but visible is usually larger than one which is not visible. Thus these criteria of visibility and palpability were used together in assessing the degree of enlargement.

All the observers concerned in the surveys reported in the present work used the above classification of thyroid enlargement. Similar standards were reached by examination of many hospital patients, before the surveys were started, and measurements of observer error were made during the course of the surveys. Representative photographs of the various degrees of thyroid enlargement are shown in Figs. 3 - 18. It should be emphasised that a general principle adopted was that if there were any doubt

Surname .....

SHEFFIELD UNIVERSITY
DEPARTMENT OF PHARMACOLOGY

Christian names .....

Card completed by .....

Date .....

Serial No. .....

Male/Female | | Age last birthday .....

Single/Married/Widowed/Divorced/Other

Main occupation during life :—

(N.B. *If female under 18 years give occupation of father ; if married give occupation of husband*).

Name of actual job, e.g., *labourer, clerk, foreman, etc.*	**Industry, section or dept.** *e.g. brickyard, shop, foundry.*
.....	.....

Patient's address .....

.....

No. of years in this district .....

Previous residential history (5 year periods up to age 30 years, then 10 year periods, giving ages) .....

.....

.....

.....

.....

Always Council water supply ?..... If not, please give details .....

.....

Any other notes .....

.....

	0—10	10—20	20—30	30—50	50+
Code					

Fig. 1. Card used in survey of thyroid enlargement in Sheffield and Ormiston

HISTORY	Yes/Doubt/No		
Any thyroid trouble ?	1	2	3
If "Yes" or "Doubtful" :			
Present goitre < 5 years	1	2	3
Present goitre > 5 years	1	2	3
Previous adult goitre	1	2	3
Adolescent goitre	1	2	3
Thyrotoxicosis	1	2	3
Myxoedema	1	2	3
Cretinism	1	2	3
Thyroid Carcinoma	1	2	3
Other	1	2	3
Any previous treatment ?	1	2	3
If so :			
Thyroidectomy	1	2	3
Oral iodine	1	2	3
Antithyroid drugs	1	2	3
Radioactive iodine	1	2	3
X-ray treatment	1	2	3
Thyroid extract	1	2	3
Other	1	2	3
Still having treatment ?	1	2	3
If so :—Oral iodine	1	2	3
Antithyroid drugs	1	2	3
Thyroid extract	1	2	3
Other	1	2	3

FAMILY HISTORY	Yes/Doubt/No		
Father with goitre ?	1	2	3
Mother with goitre ?	1	2	3
No. of brothers	No. with goitre.....		
No. of sisters	No. with goitre.....		
No. of sons	No. with goitre.....		
No. of daughters	No. with goitre.....		
No. of other blood relations with goitre—			
Male.....	Female.....		

EXAMINATION (Ring one number in each row)				
Obese	Plump	Normal	Thin	Wasted
1	2	3	4	5
Thyroid :	Not visible		Visible	Obvious
	1		2	3
Impalpable	Up to 40G	41—60G	61—80G	80G+
0	1	2	3	4
If palpable :	Unilateral			Bilateral
	1			2
,, ,,	Soft		Firm	Hard
	1		2	3
,, ,,	Smooth		Uninodular	Multinodular
	1		2	3

DIAGNOSIS (Underline one). 1. Normal 2. Diffuse goitre, 3. Multiple nodular goitre. 4. Solitary nodular goitre. 5. Other (specify) .....

Fig. 2. Card used in survey of thyroid enlargement in Sheffield and Ormiston

Fig. 3. Classification of thyroid enlargement. Gland visible but less than 40 g. Female A.W. anterior view

Fig. 4. Classification of thyroid enlargement. Gland visible but less than 40 g. Female A.W. lateral view

Fig. 5. Classification of thyroid enlargement. Gland visible but less than 40 g. Female J.B. anterior view

Fig. 6. Classification of thyroid enlargement. Gland visible but less than 40 g. Female J.B. lateral view

Fig. 7. Classification of thyroid enlargement. Gland visible but less than 40 g. Female I.W. anterior view

Fig. 8. Classification of thyroid enlargement. Apparent visible swelling but thyroid gland not palpable. Classified as not visible and impalpable. Female P.M. anterior view

Fig. 9. Classification of thyroid enlargement. Gland visible but less than 40 g. Female P.L. anterior view

Fig. 10. Classification of thyroid enlargement. Gland visible but less than 40 g. Female P.L. lateral view

Fig. 11. Classification of thyroid enlargement. Gland visible and about 40 g. Classified as less than 40 g. Female A.V. anterior view

Fig. 12. Classification of thyroid enlargement. Gland visible and about 40 g. Classified as less than 40 g. Female A.V. lateral view

Fig. 13. Classification of thyroid enlargement. Gland visible and considered to be more than 40 g., but less than 60 g. Female B.W. anterior view

Fig. 14. Classification of thyroid enlargement. Gland visible and considered to be more than 40 g., but less than 60 g. Female B.W. lateral view

Fig. 15. Classification of thyroid enlargement. Gland visible and more than 40 g., but less than 60 g. Female B.B. anterior view

Fig. 16. Classification of thyroid enlargement. Gland visible and more than 40 g., but less than 60 g. Female B.B. lateral view

Fig. 17. Classification of thyroid enlargement. Gland obvious and between 60 and 80 g. Female P.R. anterior view

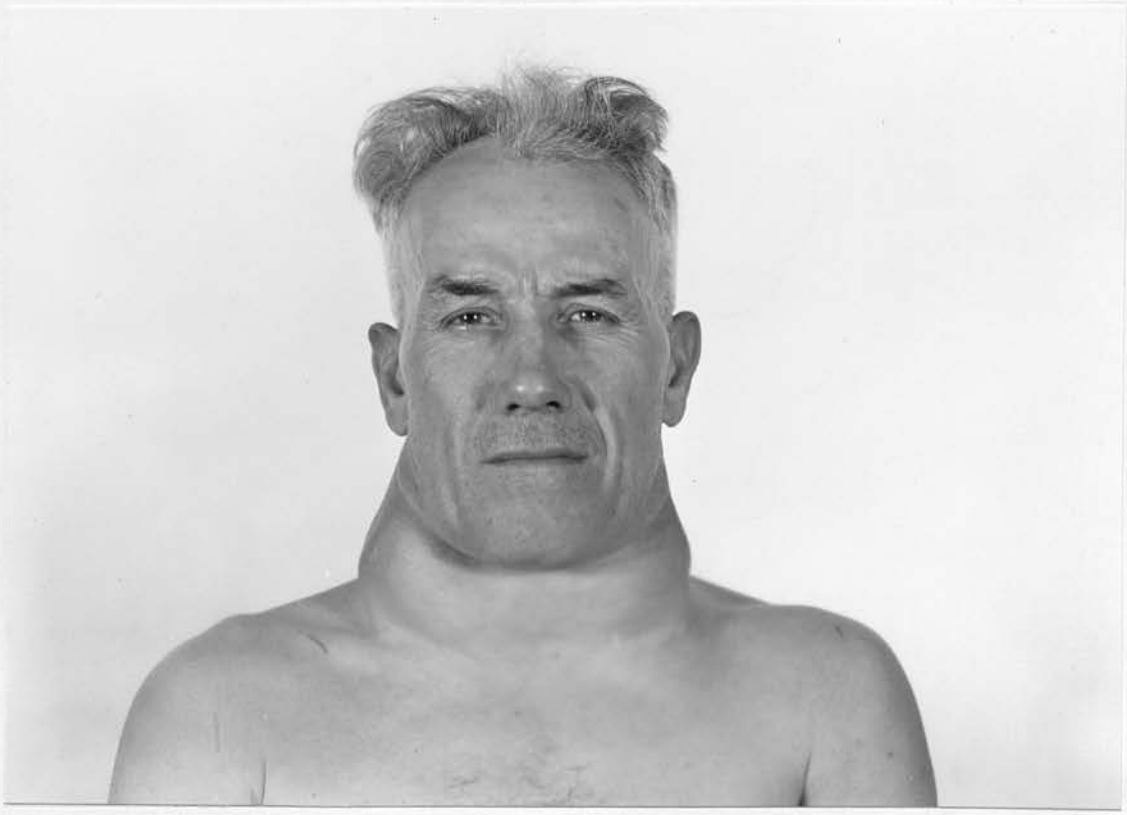

Fig. 18. Classification of thyroid enlargement. Gland obvious and more than 80 g. Male A.L. anterior view

about the gland being visible or palpable, it was recorded as non-visible or impalpable. The majority of thyroid glands are impalpable and the isthmus can barely be felt. Palpable glands were recorded when the thyroid lobes or isthmus were definitely felt.

Radio-iodine measurements

1) In vivo measurement of radio-iodine. The thyroid content of ^{131}I was measured using the method described by Brucer (48). He has shown that the most accurate method is to use a scintillation spectrometer, and the apparatus used is shown in Figs.19,20.The distance between the centre of the sodium iodide crystal and the skin was 30 cm. Inverse square law was obeyed with this counter between 10 cm. and 50 cm. The area "seen" by this counter at 30 cm. distance with the attached lead housing had a diameter of 15 cm. Thus even very large thyroid glands were within the sensitive area. The counter had lights attached to the lead and set so that their light beams crossed at 30 cm. from the crystal. When these beams coincided on an ink-spot, which was marked over the centre of the thyroid isthmus, the counter was then at the required 30 cm. counting distance. The ^{131}I standard contained in a standard size bottle,was measured in a phantom neck, of the type described by Brucer (88). The spectrometer was calibrated with this standard daily,

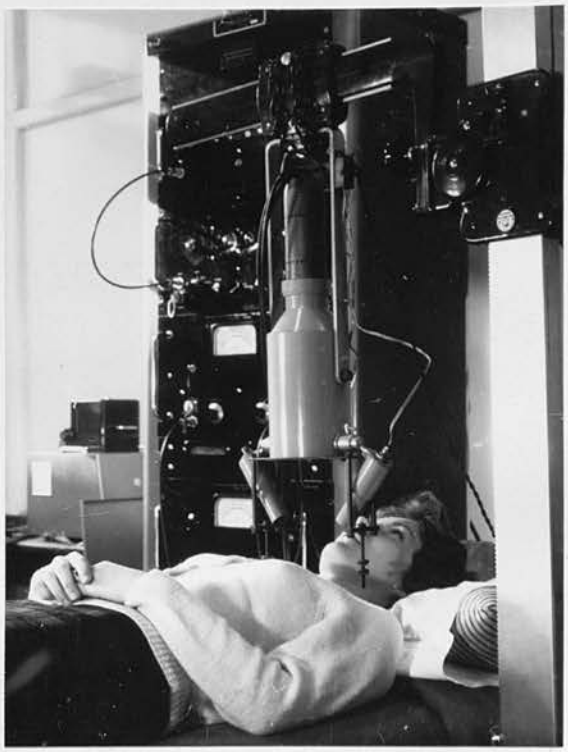

Fig. 19. Scintillation spectrometer used in measurement of thyroid gland uptakes, showing adjustable scintillation crystal assembly and electronic counting gear.

Fig. 20. Close-up of adjustable scintillation crystal assembly, showing light system used in finding fixed distance of crystal from the thyroid gland.

so that the position of the gamma photo-peak was checked. The resolution achieved with this counter is shown in Fig. 21. The peak was hardly affected by thin lead shielding between the counter and the neck, but this reduced scattered radiation considerably, and also reduced background counts. Thus the sensitivity was increased by this lead shield. The electronic gear was then set to count all of the ^{131}I photo-electrons, usually with a gate width of 10 volts. The reproducibility was within 2% and the accuracy measured on a mock glass thyroid contained within a model neck, was within 3% of the real value. All measurements were corrected for radioactive delay and expressed as percentage of the dose administered. Each dose was also measured in the phantom neck. The routine dose to patients was 10 µc, but for the measurement of total thyroidal iodine doses of 200 - 500 µc. were used, as the measurements were continued for 10 days. Efficiency of the counting system at 30 cm. was 4×10^2 counts/min./µc. ^{131}I, with a background of 58 counts/min.

2). Protein-bound radio-iodine in plasma or serum. The method of Chaikoff et al. (62) was used for the measurement of plasma protein-bound ^{131}I, 48 hrs. after giving the dose. This was the routine method for this estimation using trichloracetic acid as the protein precipitant. Specific

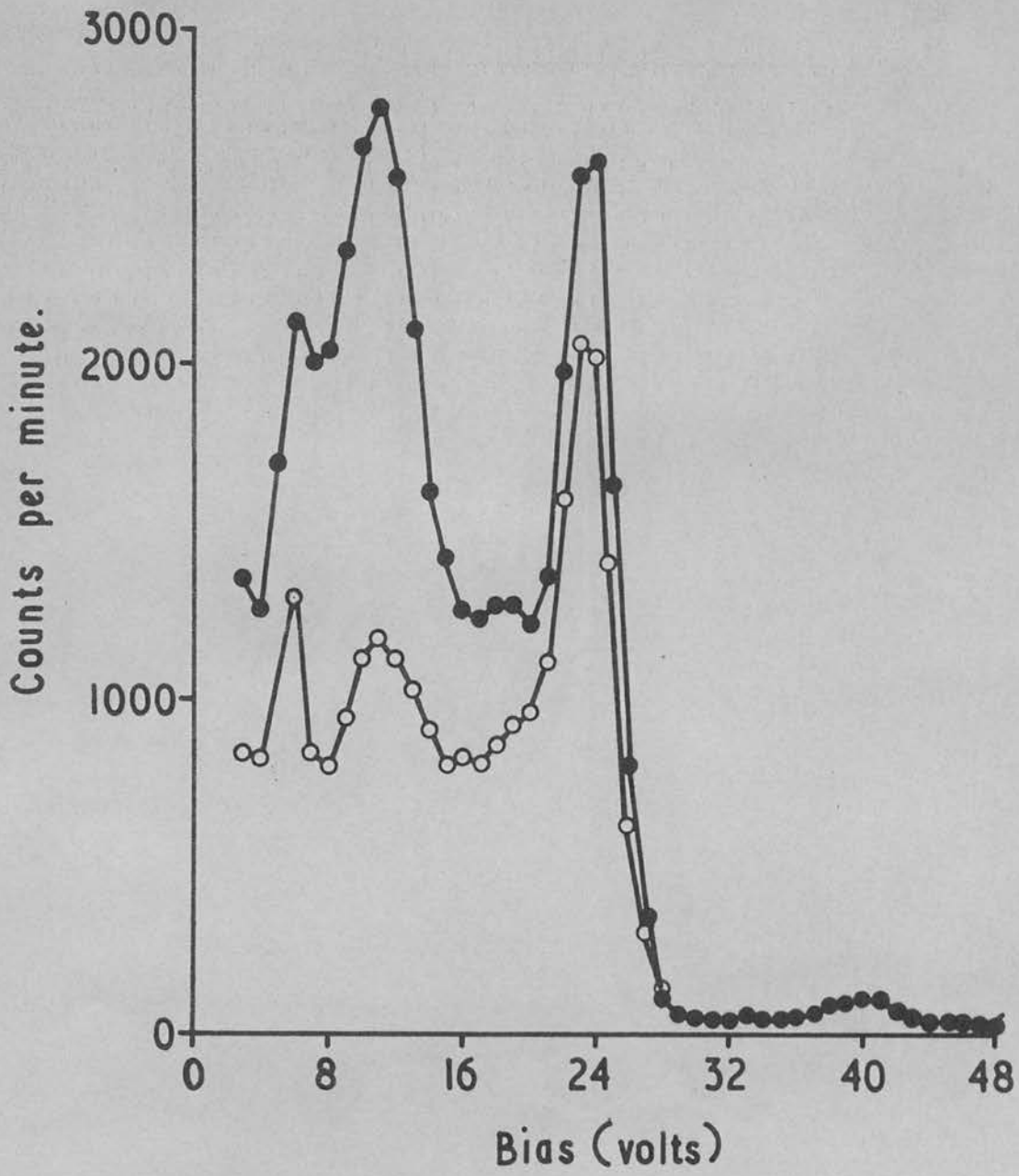

Fig. 21. Pulse analysis of ^{131}I with scintillation spectrometer shown in Figs. 19 and 20. The sharp peak at 24 v. bias is due to photo-electrons from 0·364 Me V gamma rays. The broader peak at less than 16 v. bias is due to scattered (Compton) electrons, and is much reduced by a thin shield of lead as shown by open circles. Counter was always set to count the 0·364 Me V peak.

activity values were required when total thyroidal iodine was measured, and the chemical estimation of protein-bound iodine entailed the use of zinc hydroxide as precipitant. Therefore in these studies serum protein-bound ^{131}I was measured, using zinc hydroxide as the protein precipitant. Radio-acitivity was counted in a well-type scintillation counter with pulse analysis. The efficiency was 4×10^5 counts/min./ µc. ^{131}I, with a background of 72 counts/min. All measurements were corrected for radioactive decay and expressed as % dose/litre of plasma or serum.

3). Radio-iodine content of thyroid glands after removal. Such thyroid glands were weighed, and then representative samples approximately 10gm. were weighed accurately. These pieces were incubated at 37°C. with 2N sodium hydroxide for 48 hrs. This treatment usually dissolved the gland completely, but if not more sodium hydroxide was added. The total volume was then found, and radioactivity measured in the well scintillation counter as for plasma or serum, the content being expressed as % dose in the total volume of dissolved gland.

4). Autoradiographs of thyroid glands with ^{131}I. Pieces of thyroid tissue adjoining those used for ^{131}I content, were taken for histological examination and for autoradiographs. The details of the preparation of these autoradiographs are given in Appendix 2.

Chemical estimation of iodine

a) Serum protein-bound iodine. Great care is necessary to exclude contamination of glassware when semi-micro methods for estimating iodine are used. All glassware, including syringes and needles for collecting blood were washed many times in resin-filtered distilled water. No iodine preparations were used in preparing the skin before venipuncture. A special set of syringes were kept for this particular estimation only, to exclude cross contamination with traces of blood from other patients, who might have had roentgenographic examination with organic iodine compounds.

The method used is that described by Acland ([3]), with a few modifications. It was decided to employ rigid statistical control of this method, as previous methods have had variable results. The method and the control system are described in detail in Appendix [1] , where comparison is made with other published methods. All estimations were done in duplicate, these being done in separate batches on different days. The mean difference of 457 duplicates was 0·27 µg. I/100 ml. and a mean of two duplicates in different batches was accurate to within 0·32 µg. iodine /100 ml.

b) Chemical estimation of iodine in thyroid glands. The method used was that of Kendall ([170]),

with only minor modifications, and is described in detail in Appendix 1. As much larger amounts of iodine were involved than with serum estimations, the less sensitive titration with sodium thiosulphate was satisfactory. All estimations were again done in duplicate and coefficient of variation for mean of two duplicates was 1·9%.

c) Estimation of iodine content of iodised salt. The level of iodination of iodised salt is 15 to 25 µg./gm. of salt. Thus the sodium thiosulphate titration was sufficiently sensitive. For each estimation an aqueous solution of 20 g. of salt was made and the above method for thyroid iodine followed, omitting the drying and incinerating steps.

d) Estimation of iodine content of milk

This was measured using the method described by Rodgers and Poole (296).

Experimental

a) Measurement of ^{131}I uptake. This was measured in rats following the technique described by McGinty et al., (212). A similar method was used by Searle et al., (304, 305). ^{131}I was given by intraperitoneal injection, and the dose was approximately 0·5 µc. in 0·5 ml. distilled water, without added carrier. The uptakes measured were either for 4 hr. or 24 hr. The rats were killed with chloroform

and their thyroid glands removed immediately. In the early experiments these glands were dissolved overnight in 2·0 ml. of 1 N NaOH in an incubator at 37°. The volumes were then made up to 10 ml., and counted in a Veall (356) liquid counter. Later the glands were counted whole in a fixed position within a well-type scintillation counter. This method had the advantage that the gland was still available for histological examination. In each case the ^{131}I content was expressed as % of the injected dose.

b) Chromatography of ^{131}I labelled compounds within the thyroid. The rat thyroid glands were hydrolysed with pancreatin in Krebs-Ringer-phosphate medium with added carbimazole 1 mg./ ml., at pH 7·4 for 24 hrs. Amounts varying between 5 - 20 µl. were placed on Whatman paper chromatogram strips and run in one direction with either butanol acetic acid water (75 : 10 : 15) or butanol-ethanol - 2N NH_4OH (5 : 1 : 2). Autoradiography of the strips was performed using Ilford X-ray film (Ilfex), and the paper strips counted under an end-window Geiger-Müller counter, in 1 cm. strips. The results were plotted and the areas under the curves counted by planimeter. Ratios of monoiodothyrosine to diiodotyrosine (MIT/DIT) were determined by using these planimeter readings directly.

c) Assessment of antithyroid activity. Many substances were fed to rats for prolonged periods. Apart from ^{131}I uptake described above, thyroid activity was assessed by three criteria.

Weight Gain - Body weight was measured every third day after voidance of urine and faeces.

Thyroid weight - The thyroid glands were weighed immediately after dissection on a torsion balance.

Thyroid Iodine Content This is too small in rat thyroid glands to be measured by the thiosulphate method. To each gland, 1 ml. of 4N Na_2CO_3 was added, and they were then treated as for the measurement of serum protein-bound iodine, as described in Appendix 1.

Thyroid Histology Thyroid hyperplasia was assessed histologically by a mid-section through each thyroid lobe. Assessment was performed using the same criteria and scoring system as used by Astwood (11).

d) Antithyroid activity and metabolism of thiohydantoins

Preparation of Compounds All the compounds tested have been described previously. References to the methods of preparation of 2-thiohydantoins are listed here: 5-carboxymethyl- (Johnson and Guest, 167), 5-carbamoylmethyl- and 5-indolylmethyl - (Swan, 333), 5-p-hydroxybenzyle- (Taschner, 334), 1-methyl- and 5-(4' aminobutyl) - (Elmore and Ogle, 96),

5-methylsulphonylethyl- (Elmore, Ogle and Toseland, 97), and the remainder were prepared by the method of Jackman et al. (162). Thiohydantoin acid (N-thiocarbamoylglycine) was prepared by the method of Elmore et al. (98). All compounds were chromatographically pure (Elmore and Ogle, 96). The preparations of ^{35}S-2-thiohydantoin and ^{35}S-4-carboxymethyl-2-thiohydantoin with radiosulphate are described in Appendix 3 .

Measurement of ^{35}S activity. The method was a modification of that by Bailey (25). Weighed samples of tissues, blood or urine were treated with 2 ml. of saturated solutions of Na_2CO_3 and KNO_3 and 1 ml. of saturated NaOH, and then incinerated at 600°C. for 6 hrs. The ash was dissolved in distilled water and oxidised to sulphate with 1 ml. of H_2O_2 (20 v.). The pH was then adjusted to 1·0 using thymol blue and HCl. Carrier sulphate solution (4 ml. of 0·05 N Na_2SO_4) was added and total volume evaporated in hot air oven to approximately 100 ml. 3 ml. of N Ba Cl_2 was diluted with hot distilled water and added to sulphate solution. Precipitated $BaSO_4$ was allowed to mature for 2 hr. at 95°, and then collected on filter paper by suction. These precipitates were dried overnight in a dessicator and then weighed and counted under a thin window G-M tube (E H M 2/S, G.E.C.). All counts were

corrected to a finite thickness, using a calibration chart prepared from standard solutions of radiosulphate precipitated at various thicknesses by using varying amounts of carrier sulphate. Further corrections for paralysis time and decay were also made.

Chromatography. Urine was chromatographed without dilution. Thyroid glands were homogenised, with a small amount of water, and up to 0·05 ml. of the supernatant was put on paper. Two-dimensional chromatograms were run, the two solvents being butanol saturated with H_2O and acetic acid (2000 : 1) and 1 M ethanol ammonium acetate (7·5 : 3 v/v). Collection of urine and chromatography was performed in darkness, as many of the compounds were light sensitive.

2.

SIMPLE GOITRE IN SHEFFIELD AND ORMISTON

The difficulties inherent in examining a large sample of the population in any particular region has restricted previous surveys of goitre in the United Kingdom. Usually these have been carried out on school-children, as they are localised in one site and their health a subject of concern of the education authorities. Such information is of limited value, as goitre often develops after school leaving age, and the incidence may not reflect the condition in the adult. Murray et al. (256) examined adults in Oxfordshire in 1944, but did not attempt to examine a random sample of the population.

The author was impressed by the frequency of simple goitre seen in Sheffield compared to that seen in Edinburgh. This was striking in patients referred to hospital, but these are very selected and might not reflect the general incidence. It was thought that examination of all the patients under the care of a general practitioner would give a close approximation to the incidence in the population at large. This is admittedly not an unselected sample of the population, but it is unlikely that the reasons for choosing a practitioner are related to factors producing goitre. Dr M. Rushbrooke, an experienced

female practitioner in Sheffield, agreed to examine all the patients in her practice with the aid of her assistant Dr E. Wilson. This practice draws nearly all of its patients from an area of radius approximately 3 miles in diameter and situated on the hilly west side of Sheffield, at an elevation varying between 400 and 800 ft. above sea level. The value of this survey was greatly increased by enlisting the cooperation of Dr J.S. Milne to survey his practice in the parish of Ormiston, near Edinburgh. This practice has patients from both town and country, and it was decided to limit the survey to the patients who lived in Ormiston parish. This is a small town and approximately one half of the inhabitants were in this practice. Ormiston is within 10 miles of the sea and is situated in flat farming country less than 250 ft. above sea level. These two sites of the surveys are shown in Fig.22 against the geological formation of Britain. They are both on carboniferous rock, Sheffield being mainly on coal and millstone grit, and collects water from the latter and some carboniferous limestone. Ormiston is sited entirely on carboniferous limestone.

For valid comparisons to be made, it was necessary to achieve complete examination of the two groups, and the standards for thyroid examination had to be uniform. The former was achieved within the limitation of practicability, in that 98% of the

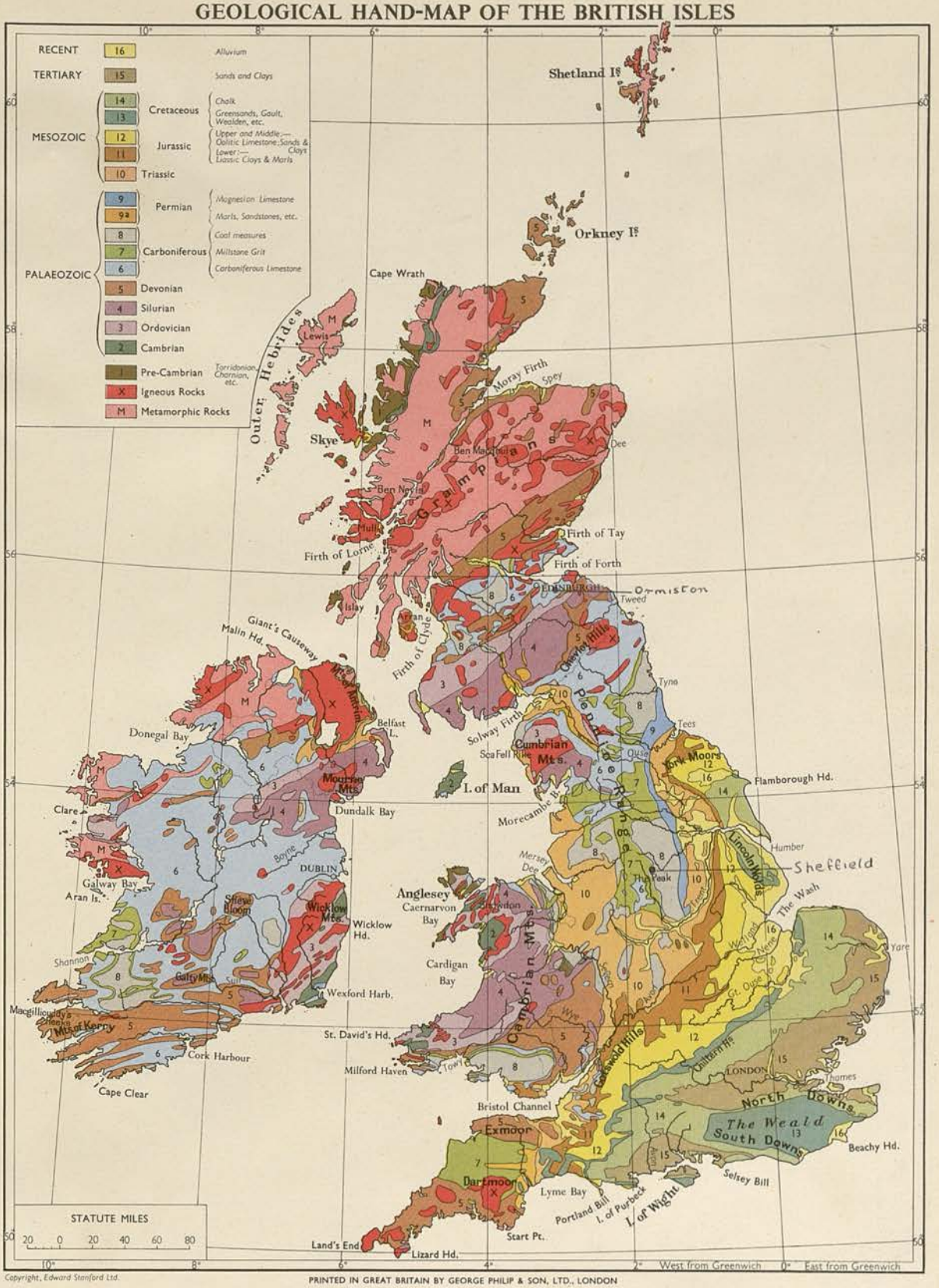

Fig. 22. Sheffield and Ormiston shown against geological formation of the British Isles.

groups were examined. This often necessitated several visits to examine elusive individuals. Uniform standards were achieved by the general practitioner and the author examining many hospital patients, with and without enlarged thyroid glands, before the surveys were begun. It was thus possible to get agreement about all degrees of the enlarged thyroid gland. The surveys were then carried out in the practices over a period of 2½ yr. and observer checks made in both by the author about 1½ yr. after the start. When each patient was examined the results were put on a standard card immediately, and details of previous thyroid disease and family history of thyroid disease were ascertained. The card used is shown in Figs. 1 & 2. The information on these cards was coded, transferred to punch cards, and sorted on an automatic sorting machine. This was not done until all the patients had been examined, so that the coding, punching and sorting operations could be performed on all the cards together.

The incidence of thyroid enlargement

The following classification was used in grouping thyroid enlargement by degree.

Normal - Thyroid not visible and impalpable.

Stage 1 - Thyroid not visible and less than 40 g. on palpation.

Stage 2 - Thyroid visible, and less than 40 g. on palpation.

Stage 3 - Thyroid visible, and more than 40 g. on palpation.

The age and sex distributions of patients examined in Sheffield and Ormiston are shown in Fig. 23, and the incidences of visible thyroid enlargement by age and sex in Fig. 24. Clearly the two practices are not similar in distribution by age or sex. There are many more women than men in the Sheffield practice, and this is probably due to being under the care of female general practitioners. In Ormiston sex ratio is almost unity, and age distribution is similar in both sexes. The age distribution in Sheffield of males is like that for both sexes in Ormiston, but the female age distribution is reversed so that there are more old women than young. This difference is unfortunate, but probably does not vitiate the value of the results.

The incidences and percentage incidences of thyroid enlargement in Sheffield and Ormiston are given in Tables 1 & 2. Thyroid enlargement above 40 g. is always easily visible and is noticed by the untrained layman. Thus the striking threefold increase in this degree of goitre in Sheffield compared to Ormiston is quite unequivocal, and is very significant on chi-square testing, $X^2 = 7{\cdot}89$ and $P < 0{\cdot}005$. Possibly even more striking is the lack of any such goitres in men in Ormiston, compared to 1·1% in Sheffield. This difference is increased to about twelvefold for thyroid glands which are visible, either in the normal position of the neck

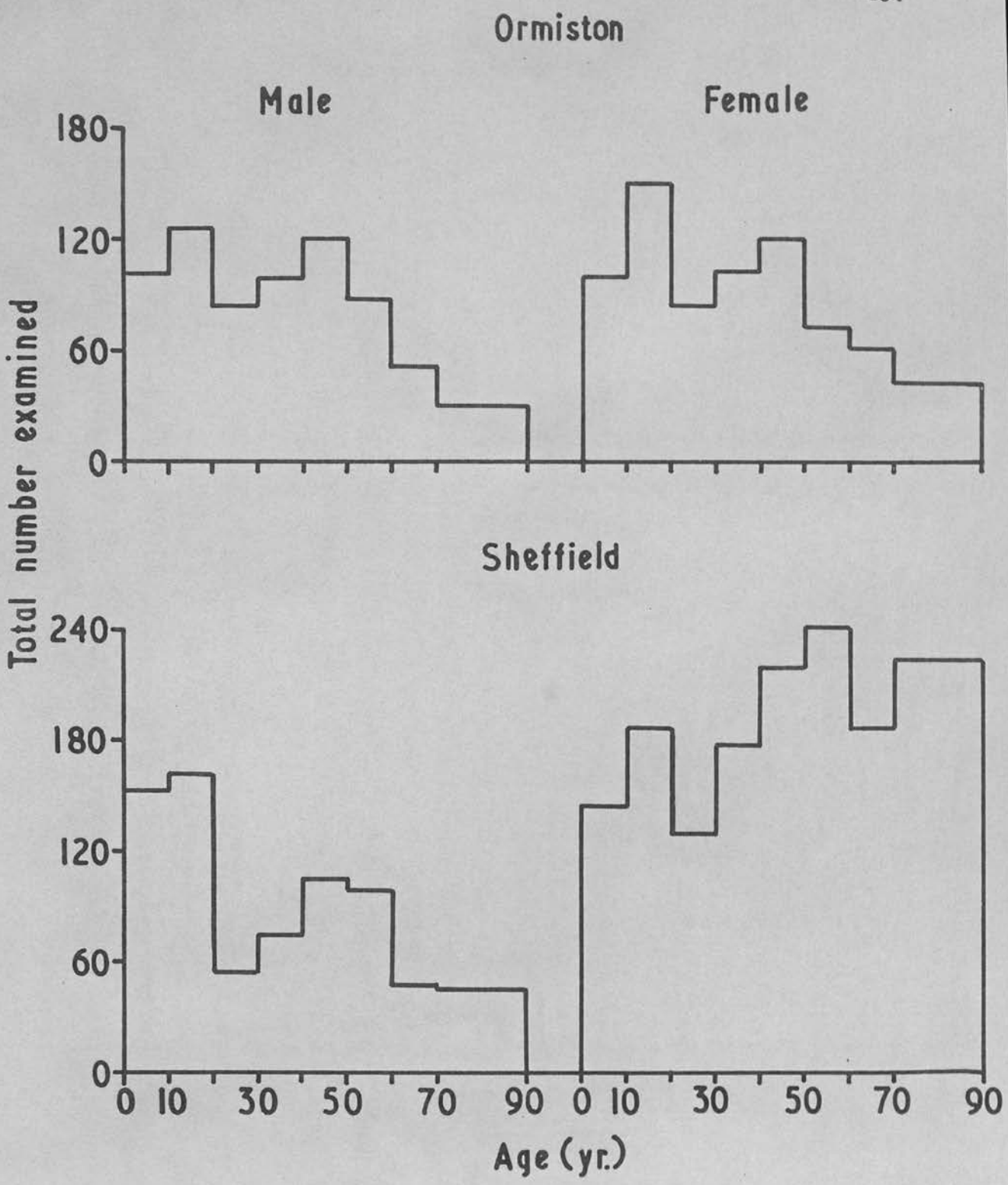

Fig. 23. Distribution by age and sex of all patients examined in the general practices in Ormiston and Sheffield.

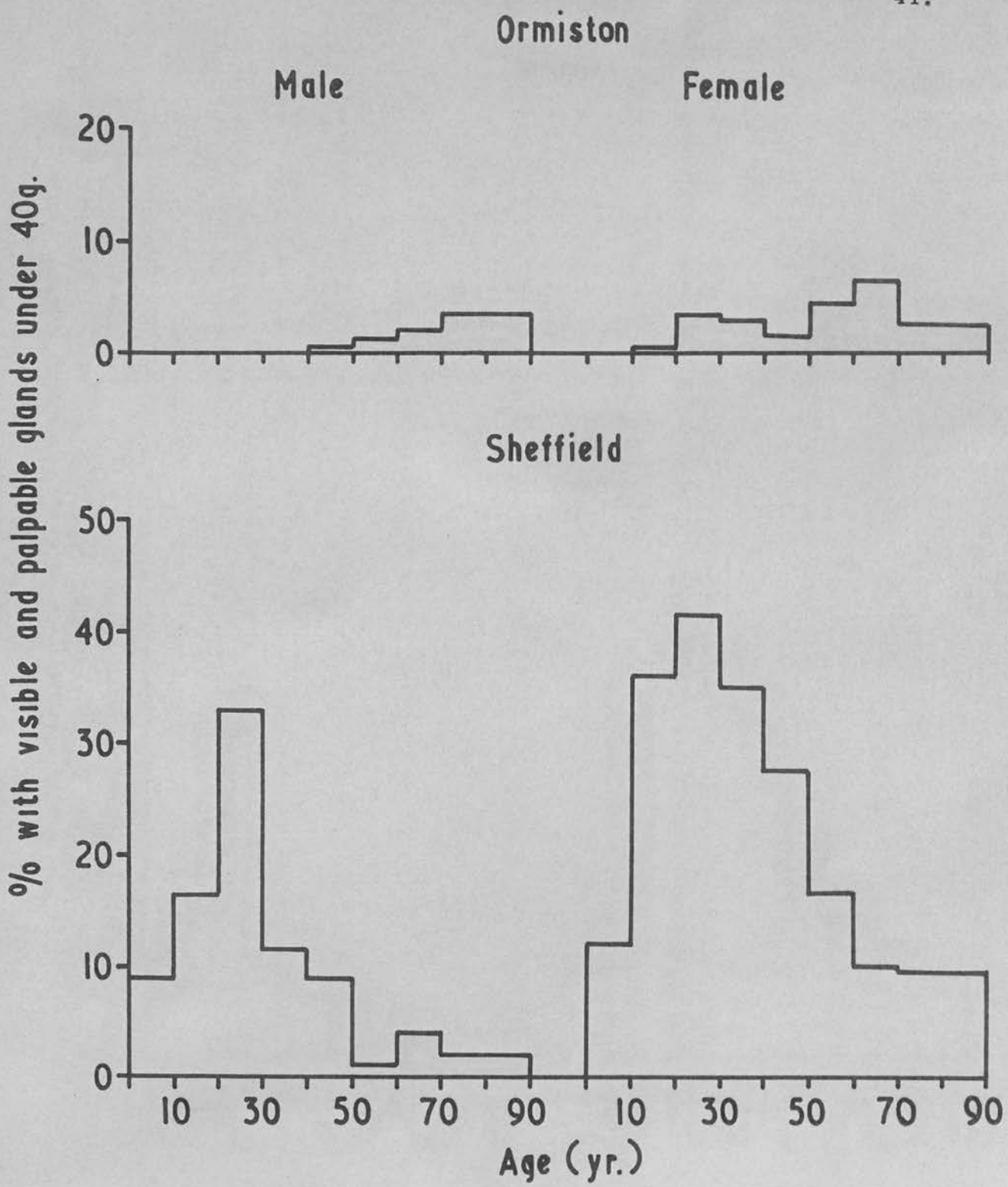

Fig. 24. Percentage distribution by age and sex of patients whose thyroid gland was visible, but less than 40 g. on palpation, in the general practices in Ormiston and Sheffield.

Table 1. Incidences of thyroid enlargement in two general practices in Ormiston and Sheffield, classified by degree, sex and age

No. of patients examined in Ormiston		No. of patients examined in Sheffield		No. of patients whose thyroid was palpable but less than 40 g. and was not visible				No. of patients whose thyroid was palpable but less than 40 g. and was visible				No. of patients whose thyroid was more than 40 g. on palpation and was visible			
				Ormiston		Sheffield		Ormiston		Sheffield		Ormiston		Sheffield	
Male	Female	M	F	M	F	M	F	M	F	M	F	M	F	M	F
101	98	154	146	2	1	62	57	0	0	14	18	0	0	0	0
126	150	162	186	10	19	91	87	0	1	27	67	0	4	4	5
84	83	57	130	6	8	26	49	0	3	19	54	0	1	2	1
97	102	77	177	7	8	47	72	0	3	9	62	0	2	0	9
120	118	107	218	9	17	60	95	1	2	10	60	0	3	0	12
86	72	100	238	1	7	54	117	1	3	1	40	0	1	0	2
51	59	49	186	1	7	21	82	1	4	2	18	0	0	1	4
30	42	48	221	2	4	13	80	1	1	1	20	0	1	1	8

Table 2. Percentage incidences of thyroid enlargement in two general practices in Ormiston and Sheffield, classified by degree, sex and age.

	Percentage of age groups of each sex in Ormiston		Percentage of age groups of each sex in Sheffield		% in each age group whose thyroid was palpable but less than 40 g. and was not visible				% in each age group whose thyroid was palpable, but less than 40g. and was visible				% in each age group whose thyroid was more than 40 g. on palpation and was visible			
					Ormiston		Sheffield		Ormiston		Sheffield		Ormiston		Sheffield	
	Male	Female	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	14·5	13·5	20·4	9·7	2·0	1·0	40·2	39·0	0·0	0·0	9·1	12·3	0·0	0·0	0·0	0·0
9	18·1	20·7	21·5	12·4	8·0	12·7	56·2	46·8	0·0	0·7	16·7	36·0	0·0	2·7	2·5	2·7
9	12·1	11·5	7·6	8·7	7·1	9·6	45·6	37·7	0·0	3·6	33·3	41·5	0·0	1·2	3·5	0·8
9	14·0	14·1	10·2	11·8	7·2	7·8	61·0	40·7	0·0	3·1	11·7	35·0	0·0	2·0	0·0	5·1
9	17·3	16·3	14·2	14·5	7·5	14·4	56·1	43·6	0·8	1·7	9·3	27·5	0·0	2·5	0·0	5·5
9	12·4	10·0	13·3	15·9	1·2	9·7	54·0	49·2	1·2	4·2	1·0	16·8	0·0	0·4	0·0	0·8
9	7·3	8·1	6·5	12·4	2·0	11·9	42·8	44·1	2·0	6·8	4·0	9·7	0·0	0·0	2·0	2·2

or with the head extended, but are smaller on palpation than glands which are considered to be over 40 g. in weight. Such glands would correspond to a grade between grades 1 and 2 given by Perez, Scrimshaw and Munoz (268), which are differentiated by visibility and not size on palpation. Examples of this degree of enlargement have already been shown in Figs. 3-12. The distribution of this degree of enlargement by age is also different in the two places, as shown in Fig. 24. In both males and females the peak incidence in Sheffield is in the age group 20 - 29 yr., but considerably higher in women than in men. In Ormiston, the incidence slowly increases with age in both sexes up to 70 yr., and again is more frequent in women. The sex ratio of incidence is about 2 : 1 in favour of women in Sheffield, and 4 : 1 in Ormiston. The percentage incidences again increase in both places for thyroid glands which are palpable but not visible. The sex ratio falls in both, and becomes unity in Sheffield. The difference between the localities also falls to about sixfold. It seems reasonable to suggest that there is a much higher incidence of thyroid enlargement in Sheffield compared to Ormiston which is most striking for glands which are only mildly enlarged, but is present for all degrees. The significance of glands which are enlarged only on palpation is

debatable, but they are different from a normal gland which cannot be felt, even in a thin neck. Similarly a normal thyroid isthmus can barely be detected, and is easily distinguished from a palpable isthmus, where there is a greater volume of thyroid tissue overlying two or three rings of the trachea.

Observer error in the surveys of Sheffield and Ormiston

When about 75% of the patients had been examined, approximately 100 patients in each practice were examined by the author (R.K.). This was done without foreknowledge of their previous examination, and the patients were unselected, apart from the selection involved in whether they were at home or not at the time the visit was paid. The results of the independent examinations are shown in Tables 3 & 4. Manifestly there is greater variation between observers and author in Sheffield. However there is a much higher incidence of palpable thyroid glands in Sheffield, and it is reasonable to expect differences in classifying these mildly enlarged glands, compared to a region where most of the glands are normal and impalpable. There was agreement in about 80% in Sheffield and 93% in Ormiston, accepting total incidences as the criteria for agreement. In Sheffield there was disagreement in 14% on differentiating between palpability and impalpability, and in 8% between non-visibility and visibility. The

Table 3. Observer error in 98 patients in Ormiston examined independently by J.S.M., and R.K.

Degree of thyroid enlargement found by J.S.M.	Degree of thyroid enlargement found by R.K.			
	Thyroid impalpable and not visible	Thyroid palpable < 40 g. and not visible	Thyroid palpable < 40 g. and visible	Thyroid palpable > 40 g. and visible
Thyroid impalpable and not visible	83	2	-	-
Thyroid palpable < 40 g. and not visible	1	8	1	
Thyroid palpable < 40 g. and visible	-	-	2	-
Thyroid palpable > 40 g. and visible	-	-	-	1

Table 4. Observer error in 105 patients in Sheffield examined independently by either M.R. or E.W., and R.K.

Degree of thyroid enlargement found by M.R. and E.W.	Degree of thyroid enlargement found by R.K.			
	Thyroid impalpable and not visible	Thyroid palpable < 40 g. and not visible	Thyroid palpable < 40g. and visible	Thyroid palpable < 40 g. and visible
Thyroid impalpable and not visible	30	6	2	-
Thyroid palpable 40 g. and not visible	9	34	1	-
Thyroid palpable 40 g. and visible	3	3	16	-
Thyroid palpable 40 g. and visible	-	-	1	-

corresponding results in Ormiston were 3% and 1%. In Sheffield the incidence of visible glands was 23% by M.R. and E.W. and 20% by the author (R.K.). In Ormiston these results were 3% (J.S.M.) and 2% (R.K.). The fact that these incidences are not significantly different from those found in the complete practices, is strong evidence that the groups examined for observer error were unbiased. It seems reasonable to conclude that the standards for thyroid enlargement had not changed significantly from the beginning of the surveys, and that it is extremely improbable that observer error could account for the differences between the two places.

Thyroid disease and treatment in the two places

Some very striking differences are seen when the findings derived from personal histories of the patients are compared, and are shown in Table 5. On a percentage basis there are three times as many patients in Sheffield compared to Ormiston, who consider that they have a goitre, present in most for more than 5 yrs. The difference is nearly five-fold for those giving a history of goitre in adolescence. Only a clear-cut history of hyperthyroidism, with either medical or surgical treatment, was accepted, and it is most striking that there were no cases in Ormiston, compared to 16 in Sheffield. Since the surveys were completed, one woman in

Table 5. History of previous thyroid disease and treatment compared by presence of thyroid enlargement within Sheffield and Ormiston, and between them.

History	Sheffield			Ormiston		
	Total 2254	No goitre 1783	goitre 471	Total 1419	No goitre 1386	goitre 33
Present goitre for 5 yr.	32**	6	26***	3	1	2
Present goitre for 5 yr.	49**	4	45***	13	4	9**
Previous adult goitre	14	9	5	5	3	2
Previous adolescent goitre	68**	25	43***	10	6	4
Thyrotoxicosis	16***	10	6	0	0	0
Previous thyroidectomy	14*	10	4	6	5	1
Oral iodine	18**	8	10**	3	1	2
Antithyroid drugs	3	2	1	0	0	0
X-radiation	6	3	3	3	2	1
Thyroid extract	18**	8	10**	3	1	2
Total with previous treatment	59**	31	28***	15	9	6

The comparisons are shown statistically within sites in col. 4 and 7, and between sites in col.2, and the level is indicated in the usual way, after testing by chi-square.

** $P < 0{\cdot}01$; * $0{\cdot}05 > P > 0{\cdot}01$; N.S. $P > 0{\cdot}05$.

Ormiston has developed thyrotoxicosis, but this still results in a highly significant difference between these two places. Differences of the same order are found for previous thyroidectomy and previous treatment with either oral iodine or thyroid extract. In Sheffield all of these incidences were significantly more frequent in those who had an enlarged thyroid on examination, except thyrotoxicosis and thyroidectomy. As both of these conditions often result in reduction of thyroid size following treatment, this seems not unreasonable. In Ormiston the numbers are too small to expect significant differences.

Family history of goitre in the two places

Results of enquiry about family history of goitre are shown in Table 6. The interpretation of these results is difficult as they are at variance. Significantly more patients with goitre in Sheffield gave a maternal history of goitre than those without goitre. This was not so for Ormiston, but the total incidence of a maternal history of goitre was significantly higher than in Sheffield ($P < 0.05$). The totals for family history of goitre in parents, siblings and children was 287 in Sheffield and 241 in Ormiston. This is a significantly higher percentage in Ormiston on chi-square testing, $X^2 = 10.64$ and P 0.01. However the

Table 6. Incidence of history of goitre in relatives, compared by presence of thyroid enlargement within Sheffield and Ormiston, and between them.

Relative	Sheffield			Ormiston		
	Total	No goitre	Goitre	Total	No goitre	Goitre
	2254	1783	471	1419	1386	33
Father	13	7	6	10	9	1
Mother	125*	87	37*	115	111	4
1 Brother	16	11	5	16	14	2
1 Sister	86	64	22	61	55	6
2 Sisters	9	4	5	4	4	0
1 Son	4	4	0	5	5	0
2 Sons	2	1	1	0	0	0
1 Daughter	31	24	7	26	24	2
2 Daughters	1	1	0	4	3	1
Other Relatives	290*	223	67	84	82	2

The comparisons are shown statistically within sites in col. 4 and 7, and between sites in col.2, and the level is indicated in the usual way, after testing by chi-square.

** $P < 0.01$; * $0.05 > P > 0.01$; N.S. $P > 0.05$.

incidence of goitre in other blood relations was higher in Sheffield, but cannot be interpreted, as the total number of other blood relations at risk was not ascertained, and may have been higher in Sheffield. Within Sheffield the total incidence of familial goitre in parents, siblings and children was significantly higher in patients with goitre ($P < 0.05$). This was not so in Ormiston, but the number of patients with goitre is too small for valid comparison.

Another method of studying the familial incidence of goitre is to use the findings on examination of complete families in the two practices. When a complete family was found on coding the cards before punching, the findings of thyroid examination for the family were noted. There were 181 complete families in Sheffield and 129 in Ormiston. Such a mass of information is rather indigestible, and some unbiased selection has to be made, taking into account that thyroid enlargement is uncommon before 12 yr. and is commoner in females. The findings in Sheffield of mother, father and 1st daughter, who was 12 yr. or over, in all families where these conditions were met, are shown in Table 7. There were 66 such families in Sheffield. Clearly there is no evidence for a familial occurrence of thyroid enlargement and shows a slight and insignificant

Table 7. Familial incidence of thyroid enlargement in families examined in Sheffield

Parents	First daughter 12+ yr. thyroid visible and palpable	First daughter 12+ yr. thyroid not visible or palpable
Both with visible and palpable thyroids	1	1
Father only with visible and palpable thyroid	1	3
Mother only with visible and palpable thyroid	7	10
Neither with visible and palpable thyroid	21	22
Total	30	36

negative correlation. This selection discards many families, but some selection is unavoidable to obtain sizable groups for comparison. It is extremely unlikely that the information from the other families could produce a positive correlation. These results are at variance with the positive correlation of family history of goitre with goitre present in the patients. One possible interpretation is that awareness of having goitre makes them more likely to remember instances of goitre in relatives, and that there is no familial incidence of goitre in Sheffield. Little information on this point can be obtained from the Ormiston families, as the incidence of goitre is so low, but there is no tendency for family history of goitre to be given only by those having thyroid enlargement.

Discussion

All goitre surveys are bedevilled by the difficulty in achieving a uniform classification of thyroid enlargement, and comparative incidences of goitre by different observers have had little meaning. The main difficulty has been in deciding the degree of enlargement which is certainly abnormal. This in turn depends on the size of the normal thyroid gland. Kloeppel (180) in 1910, found the thyroid gland in the adult Central European to weigh 34 - 41 g., whereas in 1922, Marine (227)

reported that the ratio of thyroid weight to body weight was less than 0·35 g./kg. Thus a man of 70 kg. had a thyroid of 24·5 g. Aschoff (8) studied the relationship of thyroid weight to age, and found the maximal weight to be 25 g. The normal thyroid was found to be between 20 and 25 g. by many other workers (163,260,261,207,289). McCarrison (205) confirmed the constant ratio to body weight found by Marine, but claimed that this ratio increased temporarily at puberty. De Smet (88) measured normal thyroid weights in the Belgian Congo and found a normal weight of 20 - 25 g. in adults. Sigurjonsson (311) in Iceland found the normal thyroid weight to be 14 g. in men and less in women, this being a region where iodine intake is probably high. Clearly the normal thyroid weight is usually 20 - 25 g. and may be less than this in certain regions. Hazard and Kaufman (142) considered that any gland over 35 g. was abnormal. This same arbitrary weight was used by Hull (159), and in Colorado he found 21·3% of thyroid glands to be enlarged. Many arbitrary standards of abnormality have been used, when only clinical examination has been done. Murray et al. (256) accepted visibility as the criterion for enlargement, as did the Study Group on Endemic Goitre of the World Health Authority (332), but the former group accepted as abnormal any gland which

could be seen, whereas the latter group only accepted an obvious gland as Stage 1 goitre, and such enlargement is about 80 - 100 g. Clearly the results of surveys by these two groups cannot be compared. Perez et al. (268) reviewing the technique of surveys, emphasised the importance of glands which were enlarged only to palpation and were not visible. These workers classify such glands as Grade 1 goitre, and claim that they are 4 or 5 times the size of a normal gland, which would mean between 80 and 100 g. This cannot be reconciled with the W.H.O. classification. However Perez et al. state "usually a thyroid gland whose lateral lobes have a volume greater than the terminal phalanges of the thumbs of the person being examined will be considered goitrous." The average volume of such phalanges in the adult is 20 ml. Evidently they have considered goitrous glands which are palpable and weigh between 25 - 35 g. in adults. In the present work, all palpable and visible glands were recorded. The definition of normal and abnormal has been avoided, but clearly the majority of glands cannot be palpated or seen. As the normal gland is about 20 - 25 g., it is reasonable to assume that palpable glands were heavier than this. A visible gland is usually more enlarged and a gland becomes visible about 35 - 40 g. in an average neck. It is unlikely that survey results can be compared whenever arbitrary divisions

between normality and abnormality are used.

Geographical differences found by the same observers are much more valid than those between different surveys. In 1838 Inglis (161) noted striking differences in incidence of goitre in different parts of England, and very small incidences in Scotland. This was confirmed by Roberts (293), and a detailed account was given by Berry (33) in 1901. In 1924 all school-children aged 12 yr. in England were examined at the request of the Board of Education and the results were published by Campbell (55) and in more detail by Stocks (331). Unfortunately this survey has doubtful significance as no standards were given to the school medical officers. The resulting observer error was checked by Stocks (331) and found to be very large. Sheffield was reported as an area of moderate prevalence of goitre, with an incidence of 4·5% in girls. This large survey confirmed that the ratio of girls to boys with goitre always fell to unity in all areas with a high prevalence of goitre. Striking differences in goitre incidences in girls were found between Somerset and Suffolk by Young et al. (395) in 1936, using a similar classification to the present work and that of Perez et al. (268). In Somerset 56% of children between 6 and 14 hr. had visible thyroid enlargement and 3·7% in Suffolk. Davies and Rogers (86) noted a high incidence of goitre in adult women in South

Wales in 1940, and Lisney (195) found 15·6% of pregnant women in Dorset had thyroid enlargement in 1951. The first attempt to survey the population of one village in England was reported by Murray et al. (256) in 1948, but only 50% of the inhabitants were examined. The incidence of visible enlargement was 26%. Selected groups of adult women from other regions in England were examined by the same workers, and incidences varying from 9% to 50% were found. They also examined school-children from many regions in both England and Scotland, and found much higher incidences in England, especially in Oxfordshire and Dorsetshire. Hughes et al. (158) reported in 1959 the incidences of goitre in school-children from some areas that had been examined in 1948 by Murray et al. (256), and showed that these had not decreased. Both investigations confirmed the finding that the sex ratio of those with goitre approached unity in regions of high prevalence. The present results from Sheffield and Ormiston confirm that striking regional difference in incidence of thyroid enlargement still occur, but it is not possible to compare the incidences with those previously reported from various parts of England and Scotland, due to the reasons given above. The decrease in sex ratio from 4 : 1 in Ormiston to 2 : 1 in Sheffield also confirms previous observations. The magnitude of observer variation indicates that, though this

cannot account for the regional differences the observed incidences are only approximations. Thus it is entirely possible that other observers might find the incidence in Sheffield seven times that in Sheffield, compared to the twelve times for visible goitre under 40 g. reported here. The regional differences in history of thyroid disease seem to reflect the observed difference in goitre incidences, and the increased number of thyroidectomies in Sheffield is also an indication of this. It is possible that such a difference might be due to selection for such treatment, but it seems probable that it would be recommended more often in a low goitre area, because of the possibility of thyroid carcinoma. The very striking difference in incidence of hyperthyroidism is exceedingly important, and seems entirely unequivocal. Campbell (55) using death rates for thyrotoxicosis showed an association between incidence of this condition and simple goitre in England and Wales. As death from hyperthyroidism is uncommon it is unlikely that the incidence of hyperthyroidism is accurately indicated by its death-rate, and this demonstration is very indirect. Similar results have been reported from North and South America (207,269), Finland (300), and Australia (394, 69). No association between these conditions has ever been reported from Switzerland

or the Himalayas, where goitre was prevalent. Direct evidence for such an association is given in the present work, but no causal relationship can be assumed. If this were shown, it would be of great importance in elucidating the aetiology of simple goitre.

The conflicting results from Sheffield concerning familial incidence of goitre underline the difficulties of investigations of familial conditions. Familial incidence of simple goitre has been usually assumed to occur (127), but little examination of complete families has been done. An exception to this was the study of twins in Switzerland by Eugster (99), which showed a common environmental cause and not a genetic one.

Summary

1. A survey of thyroid enlargement has been carried out in two general practices, one in Sheffield and the other in Ormiston, East Lothian.

2. The incidence of visible thyroid enlargement above 40 g. was 2·2% in Sheffield and 0·8% in Ormiston. The corresponding rates for visible enlargement below 40 g. was 18·7% and 1·5%.

3. Personal history of goitre, past or present, was significantly more frequent in Sheffield, and there were twice as many thyroidectomies in Sheffield.

4. Sixteen cases of hyperthyroidism had occurred in the population in Sheffield, but none in Ormiston.

5. There was no evidence of a familial incidence of goitre in either place.

3

THE EFFECT OF SUPPLEMENTARY IODINE ON SIMPLE GOITRE IN SCHOOL-CHILDREN IN OXFORDSHIRE AND WILTSHIRE

The Goitre Subcommittee of the Medical Research Council recommended in 1944 (121) and again in 1948 (256) that iodized salt should be made available throughout the United Kingdom, as a prophylaxis against the development of simple goitre. This has not been implemented so far. However the high goitre incidences found by Murray et al. (256) in 1948 and Hughes et al. (158) in 1956 in school-children resulted in the introduction of iodized salt in all schools in Oxfordshire and Wiltshire. This was done on the recommendation of the county school medical officers, and was begun between 1957 and 1958. Accordingly in 1960 a further survey of thyroid enlargement in school-children from these two counties was planned. The author was given the opportunity to take part in this survey as the representative of the Medical Research Council. The other representatives were Dr W.T.C. Berry from the Ministry of Health and Dr J.N.Horne from the Ministry of Education. The same classification for thyroid enlargement was used as for surveys in Sheffield and Ormiston, and uniformity by the three observers was attempted by

examining together many school-children in London before the surveys were begun. As a result of this experience, it was decided to note separately those children, where the thyroid isthmus was easily palpable but the thyroid lobes were difficult to feel or impalpable. Such thyroid glands were included with palpable lobes in the Sheffield and Ormiston surveys. This was done in Oxfordshire and Wiltshire as children with enlarged thyroid isthmus appeared to be a distinct and large group. As in the Sheffield and Ormiston surveys any gland which was doubtfully palpable or visible was recorded as being impalpable or not visible. The surveys were restricted to girls and boys over 13 yr. in Oxfordshire and to girls between 13 and 15 yr. in Wiltshire. All such girls in every school visited were examined, except for those who were not given parental permission, and these were always less than 5%. In Oxfordshire the schools were smaller and the numbers examined were increased by examining boys. Each child was examined in turn by all three observers, and the results given are the findings of the author, except for the results on observer variation in Wiltshire. The examination was performed without the observer knowing the findings of the other two, and recorded separately.

The results from Oxfordshire

Incidences of the various degrees of thyroid enlargement found in children from five towns or villages in North Oxfordshire are given in Table 8. There are variations between these places in relation to thyroid enlargement but these are not statistically significant. When they are taken together, the sex ratio for each degree of enlargement is about 3 : 1 in favour of girls, and 4·3% of the girls had visible thyroid glands. A further 30% of the girls had palpable thyroid glands, including the group which had an easily palpable thyroid isthmus. It is striking that only 1 girl with a visible enlargement considered to be more than 40 g. was seen in all these schools. This incidence rate of visible enlargement is less than that in Sheffield, but the rates in the latter are given for the decade 10 - 19 yr., which is not directly comparable with the Oxfordshire rate, as the girls were all under 16 yr. The incidence of palpable thyroid glands is approximately the same in Oxfordshire and Sheffield. The present results are so much lower than the percentages of visible thyroid enlargement found in these same schools by Murray *et al.* (256) and Hughes *et al.* (158) that it is difficult to believe they can be compared. In 1948 they found 37% visible in Banbury, 22% in Chipping Norton, 37% in Hook Norton, 29% in Witney, 14% in Bicester and the

Oxfordshire between the ages of 13 and 15 yrs, classified by school and sex

School	Number of children examined		Number of children whose thyroid was palpable, but 40 g. and was not visible		Number of children whose thyroid was palpable, but 40 g. and was visible		Number of children whose thyroid isthmus alone was enlarged	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Banbury Grammar	-	95	-	15	-	2	-	18
Banbury Secondary Modern (1)	27	67.	0	6	0	3	2	7
Banbury Secondary Modern (2)	-	90	-	8	-	3	-	8
Banbury Technical	38	58	1	4	1	3	1	10
Chipping Norton Grammar	-	93	-	14	-	6	-	17
Hook Norton Secondary Modern	58	47	3	16	1	4	4	8
Witney Grammar	-	91	-	10	-	3	-	16
Witney Secondary Modern	-	92	-	17	-	7	-	18
Bicester Grammar	13	82	0	12	0	1	1	15
Bichester Secondary Modern	-	97	-	11	-	3	-	14
Total by sex	136	812	4 (2·9%)	113(13·9%)	2 (1·5%)	35 (4·3%)	8 (5·9%)	131 (16·1%)
Grand Total	948		117 (12·3%)		37 (3·9%)		147 (15·5%)	

mean was 26·9%. In 1958 this mean had increased to 40·4%.

Iodized table salt but not cooking salt was used in all these schools for at least 18 months, and in most for 2½ yr. before the present surveys were carried out. The salt used was iodized to a level between 1 part in 75,000 and 1 part in 40,000. Several samples were taken from the schools and analysed in Sheffield. The iodide found was always between these levels. This level means that the ingestion of 1 gm. of this salt would provide between 15 and 25 µg. of iodide. The information concerning which children took school lunch was given by the school authorities, and the children were asked about taking salt with lunch in the following way. In groups of 4, after being examined, enquiry was made about the taking of water with lunch, and the results apparently recorded, and then about the taking of table salt with their lunch. In this way, it was hoped to get a true answer to this question, without any bias towards giving an answer which they might think was fit and proper. Unfortunately this method of questioning was only started on the second day of the survey, and the number with a salt history is lower than those taking school lunch. The results are shown in Table 9 . There was no significant difference between the incidence of visible or palpable

Table 9. Thyroid enlargement in school children in Oxfordshire, classified by lunch habit and salt intake.

Thyroid state	Taking school lunch		Taking iodised table salt with school lunch	
	Yes	No	Yes	No
No. examined (948)	514	434	186	218
No. with palpable thyroid but not visible	66	51	30	21
No. with visible thyroid	24	13	12	8
Total no.with palpable thyroid	90 (17·5%)	64 (14·7%)	42 (22·6%)	29 (13·3%)

thyroid in the children taking school lunch (17·5%) and those not taking school lunch (14·7%). Rather more than half (54%) of the children taking school lunch did not take extra table salt with their meal, and this incidence of salt taking was not different from children who did not have school lunch. Of those taking salt, 22·6% had a palpable or visible thyroid, and this incidence was 13·3% in those not taking salt. This difference is significant $X^2 = 4·86$, $P < 0·05$, and is the reverse that would be expected from iodine supplementation. A similar difference applies to visible thyroid enlargement alone, but is not significant ($P < 0·25$).

The results from Wiltshire

Schools were visited from eight towns in Wiltshire, both upper Stratton and Wroughton being included in Swindon, and the incidences of thyroid enlargement are shown in Table 10 . As in Oxfordshire, there are differences between schools, but none of these reach significance. Only 2·6% of girls examined had visible thyroid enlargement, and a further 20·3% had palpable thyroid glands. Both these incidences are considerably lower than the corresponding results from Oxfordshire (4·3% and 30%) and on chi-square testing the difference for visible glands is on the borderline of significance, $X^2 = 3·46$, $P < 0·10$, and that for palpable glands is

Table 10. The incidence of thyroid enlargement in female school-children between the ages of 13 and 15 yrs. from various schools in Wiltshire

School	Number of girls examined	Number of girls whose thyroid was palpable, but 40 g. and was not visible	Number of girls whose thyroid was palpable, but 40g. and was visible	Number of girls whose thyroid isthmus alone was enlarged
Melksham	95	9	0	9
Devises	83	7	0	7
Chippenham	99	9	4	13
Corsham	86	5	2	6
Calne Grammar	91	9	2	12
Calne Secondary Modern	93	9	5	14
mesbury	93	8	6	15
urrington	97	11	2	13
roughton	42	4	0	4
pper Stratton	83	5	1	6
Total	862	76 (8·8%)	22 (2·6%)	99 (11·5%)

highly significant, $P < 0.001$. It may be important in interpreting these differences between counties, that the Oxfordshire survey was carried out at the end of March, 1960, and that in Wiltshire at the end of September, 1960.

In Wiltshire schools iodized salt has been used for cooking purposes, as well as for table use. The level of iodization was the same as in Oxfordshire, but a much larger iodine supplement would be expected from the use of the salt for cooking. It was therefore not necessary to ask the children about their use of table salt. Samples of cooking salts were analysed for iodine content by the Government Chemist before the survey was done. During the survey further samples were taken and analysed in Sheffield by the author. Some few weeks after the survey further samples of salt were taken and analysed in Sheffield, and at the same time one whole lunch was collected from each school. One of the ten lunches collected had sea fish for the main course. This is a smaller fish proportion than the average weekly content for school lunches, which is 1 fish meal in 5 meals. These ten meals were bulked, and several aliquots measured for iodine content by the Government Chemist. The results showed a mean iodine content of 120 µg. of iodine per meal. The results for iodine contents of cooking salts are given in Table 11. The levels of tolerance given by the manufacturer are 13 - 25

Table 11. The iodine content of cooking salt collected from schools in Wiltshire, and analysed independently in two laboratories

School	Iodine content of cooking salt collected Aug.1960 and analysed by M.O.H. chemist	Iodine content of cooking salt collected Sept. 1960 and analysed by R.K.	Water content of cooking salt collected Sept. 1960 %	Iodine content of cooking salt collected Nov.1960 and analysed by R.K.
Melksham	14	12	0·6	20
Devises	10	8	2·2	15
Chippenham	12	10	11·2	14
Corsham	9	0	7·2	0
Calne Grammar	16	17	9·1	33
Calne Sec. Modern	12	16	1·3	13
Amesbury	11	19	1·6	13
Durrington	11	12	9·4	8
Upper Stratton	5	15	16·2	19
Wroughton	13	-	-	-

p.p.m., and the results are mostly slightly below this lower level. The water content is variable and probably depends on how the salt has been stored. It was known that Corsham salt would show no iodine, as their supply of iodized salt had just been interrupted at the time of the survey. There is good agreement between the two laboratories except for salt from Upper Stratton.

The incidence of thyroid enlargment in Wiltshire is shown in Table 12, classified according to the taking of iodized school meals or not, and the results of the three observers are given. In Wiltshire slightly less children had visible or palpable thyroid enlargement when taking school lunches compared to those not taking them, but the differences are small and not significant. Supplementation of such meals with iodine had been carried out for at least 18 months, and in most schools for 2½ yrs, and it appears to have produced no significant reduction in thyroid enlargement. There is close agreement between observers for visible thyroid enlargement but less close for palpable thyroid glands. One observer (W.T.C.B.) consistently felt more than the other two observers, who agreed closely. However it is striking that the differences related to the taking of school lunches, were of the same order for each observer.

Table 12. Observed variation of thyroid enlargement in female school-children in Wiltshire, classified by the taking of iodised school lunch

Observer	Incidence of those taking school lunch in all girls examined		% incidence of those whose thyroid was palpable, but 40 g. and was visible, classified by school lunch taking		% incidence of those whose thyroid was palpable, but 40 g., and was not visible, classified by school lunch taking		% incidence of those whose thyroid isthmus alone was enlarged, classified by school lunch taking	
	Number taking	Number not taking	% in takers	% in non-takers	% in takers	% in non-takers	% in takers	% in non-takers
W.T.C.B.	502	360	2·4	2·8	12·9	13·1	20·7	21·4
J.N.H.	502	360	2·0	2·8	9·4	9·2	11·6	13·9
R.K.	502	360	2·2	3·1	8·6	9·2	11·0	12·5
Mean of three observers	502	360	2·2	2·9	10·3	10·5	14·4	15·9

Discussion

The discrepancy between the present results of thyroid enlargement in Oxfordshire and Wiltshire, and those given by Hughes *et al.* (158) in 1958 for these same schools is large, and emphasises the point made in the last chapter concerning the difficulty in comparing the results of surveys by different observers. A reasonable explanation is that it is due to differing standards and that glands classified as (b) "visible to the trained observer" by Hughes *et al.* (158) and before by Murray *et al.* (256), are classified by the author as palpable but not visible. It should be emphasised that whenever there was doubt about visibility, the gland was recorded as non-visible in the present work, whereas in these previous surveys emphasis is laid on noting any deviation from the normal contour of the neck. The latter approach tends to magnify goitre incidences. However, it might be reasoned that the goitre incidence in these two counties had been much reduced by increased iodine intake during the interval between the surveys. This is not likely, as iodized cooking salt is not generally available in these counties, and iodine supplementation of school meals has not significantly altered the incidence of thyroid enlargement in those children taking such meals, compared to children not taking school lunches.

In discussing any results on iodine supplementation, it is essential that such supplement has been shown to be adequate. The human daily requirement for iodine is debatable, but is unlikely to be more than 100 µg. (44,121,124,263). The analyses of school meals from Wiltshire showed that each meal contained 120 µg. of iodine. As children spend about six months of each year at school, they will get at least 60 µg. daily from this one meal. This represents a considerable part of their daily requirement. It might be argued that the intake of the children not taking school lunch, might be of the same order, from their home meals. If this is so, enlarged thyroid glands are present in Wiltshire schoolchildren, when their iodine intake is not low. Many workers regard such thyroid enlargement in school children as physiological occurring at or near puberty, and that it bears no relationship to adult goitre (205, 206). Though this is true in the sense that it represents an increased demand for thyroid hormone, and a compensatory enlargement of the thyroid to provide this, fundamentally the enlargement is a result of an inadequate supply of iodine for this increased hormone synthesis. However an inadequate supply of iodine to the gland is not necessarily due to inadequate intake of iodine. Further, there is considerable documented evidence

from many parts of the world that treatment of school-children with iodine produces a reduction in incidence of thyroid enlargement and reduction in size of enlargement already present. Marine and Kimball (232, 233) showed this many years ago in America and Brush and Altland (49) have reported recently on the results of iodine prophylaxis on the community scale in Michigan. There is also very convincing evidence from Switzerland that iodine supplementation has dramatically decreased thyroid enlargement of the newborn (Pradervand, 277), school-children (Wespi, 378) and adults (Nicod, 258). Perhaps the most convincing study is that by Scrimshaw (302) in Central America, as it was rigidly controlled and used the double-blind principle of clinical trial. Striking decreases in goitre incidence occurred but the dose of iodine used was large and corresponded to a daily consumption of salt iodized to a level of 1 part in 10,000. Even more significant was that this effect was found within 20 weeks of starting such treatment. Wespi (378) found a striking decrease within 1 year in school-children in Lausanne, and the level of iodization of salt was 1 in 200,000. Many other trials have been reported showing decrease of goitre in children (68, 106, 145, 323). There are very few reports on the lack of effect of iodine on thyroid

enlargement. Certainly the most striking is that by Clements and Wishart (71) on thyroid enlargement in school-children in Tasmania. Some other regions of the world have been found to have goitre even with high dietary intake of iodine (52, 157, 309). There are few reports from England on the effect of iodine on thyroid enlargement. Inglis (161) in 1838 showed reduction in size of goitres by the administration of iodine. Turton (345, 346) published a very full and detailed account of goitre in Derbyshire, with emphasis on thyroid enlargement in school-children. He gave iodized sweets to the children and also iodised the water-supply for a period of eleven months. The sweets given weekly provided 75 mg. of iodine per year. There was no decrease in the incidence of thyroid enlargement at the end of the trial and in some age groups it was increased. A further controlled trial for periods between 4 and 12 months again showed no decrease from iodine therapy. The amount of iodine given in Wiltshire for the present study was such that it would bring an iodine-deficient diet up to normal intake levels. It may be concluded that this supplementation produced no significant decrease in thyroid enlargement after a period of at least 18 months. It is possible that an effect may be produced if this supplementation is continued for a longer period. Another explanation of the lack of effect of

iodine is that some other factor is related to the taking of school lunches and overcomes the effect of added iodine. The difference in incidence of thyroid enlargement in Oxfordshire and Wiltshire may be explained on a geographical basis, similar to the much greater difference between Sheffield and Ormiston. Another explanation is that there is a seasonal variation, and this is suggested as Clements and Wishart (71) have noted such a variation in Tasmania, with peak incidence in the spring. This point will be considered fully in a later chapter.

Summary

1. The incidence of visible thyroid enlargment under 40 g. in size in 812 Oxfordshire school-girls was 4·3%, and a further 30% had palpable thyroid enlargement.

2. The corresponding incidences in 862 school-girls from Wiltshire was 2·6% and 20·3%.

3. Iodine supplementation by iodized table salt in Oxfordshire and iodized cooking and table salt in Wiltshire produced no significant decrease in incidence of thyroid enlargement, after administration for at least 18 months.

4

OBSERVATIONS ON IODINE METABOLISM IN SIMPLE GOITRE IN SHEFFIELD

Simple enlargement of the thyroid gland is often the only complaint of patients seeking medical attention in Sheffield. Over a period of four years, a large number of such patients was seen and investigated. Hyperthyroidism was excluded by the lack of clinical symptoms and signs apart from thyroid enlargement, by radioiodine measurements, and later by estimation of serum protein-bound iodine. Values above 0·4% dose/litre for plasma protein-bound ^{131}I, or above 7·5 µg./100 ml. for serum protein-bound iodine by chemical estimation were suggestive of hyperthyroidism. All patients were observed for many months, were weighed frequently and occasionally response to antithyroid drugs was assessed, when the diagnosis between simple goitre and hyperthyroidism was difficult. Lymphocytic thyroiditis was also excluded. All patients with thyroid enlargement had serum thymol turbidity and thymol flocculation tests performed by the hospital biochemical laboratory. Luxton (199) and Roitt et al. (297) have shown that serum flocculation tests are abnormal in nearly 90% of untreated cases of lymphocytic thyroiditis and that these tests are of diagnostic value. When these flocculation tests were abnormal, or any clinical reasons for suspecting thyroiditis e.g. very firm or hard thyroid, or

symptoms and signs of mild hypothyroidism, serum precipitation tests for the detection of thyroid auto-antibodies were performed by the biochemical laboratory at Derby Royal Infirmary.

Thyroid enlargement produced by drugs was excluded by a careful history. Patients taking any drug for three months were excluded, even though goitrogenic activity had not been demonstrated for the particular drug. Well known goitrogenic drugs are para-amino salycylic acid, phenylbutazone and iodopyrine (Morgans and Trotter, 252).

It was possible to investigate with radioiodine a similar number of patients without thyroid enlargement. These patients had been sent for a routine radioiodine tracer test to exclude hyperthyroidism. Their symptoms were usually those of anxiety and this investigation was often performed for reassurance only. It may be questioned whether such patients can be described as normal, but they have no thyroid enlargement. Normal volunteers without thyroid enlargement gave blood for serum protein-bound iodine estimation. They were selected from medical students, nurses, university staff and euthyroid patients. By selection a normal group was obtained of similar age distribution to patients with simple goitre, where protein-bound iodine had been measured.

<u>The patients from the Sheffield region</u>

Over the period of study, 240 patients were

accepted as having simple goitre, either diffuse or nodular. There were 225 women and 15 men, giving a sex incidence of 15 : 1. Clearly these patients do not represent a cross section of the community at large, as can be seen from Chapter 1. The main selecting factor was undoubtedly the size of the gland. All these patients had an easily visible thyroid, this being the main reason for attendance. Because of this, the emphasis of the study was on iodine metabolism of patients with goitre; and not on the natural history and clinical features of the goitre. Patients under the age of 18 yr. did not have studies with ^{131}I, because of the danger of radiation effects to the thyroid in young people (384).

The uptake of radioiodine by the thyroid gland

This was measured in 194 patients with simple goitre and in 180 normal subjects. The 4 hr. and 48 hr. uptakes have been grouped in 5% intervals and plotted as a frequency histogram in Figs. 25 and 26. In Fig.27 the 48 hr. uptakes have been plotted against age in 5 yr. age-groups, and the regression lines of uptake against age are drawn. The results are summarised in Table 13 . The mean 48 hr. uptake in goitre patients was 50·1% ± s.e. of mean 0·86%, and in normal subjects 43·1% ± 0·76%. A generalised t-test confirmed that these means are significantly different $F = 18{\cdot}30$ ($P < 0{\cdot}001$). A regression analysis with age as the independent variable showed no significant difference between

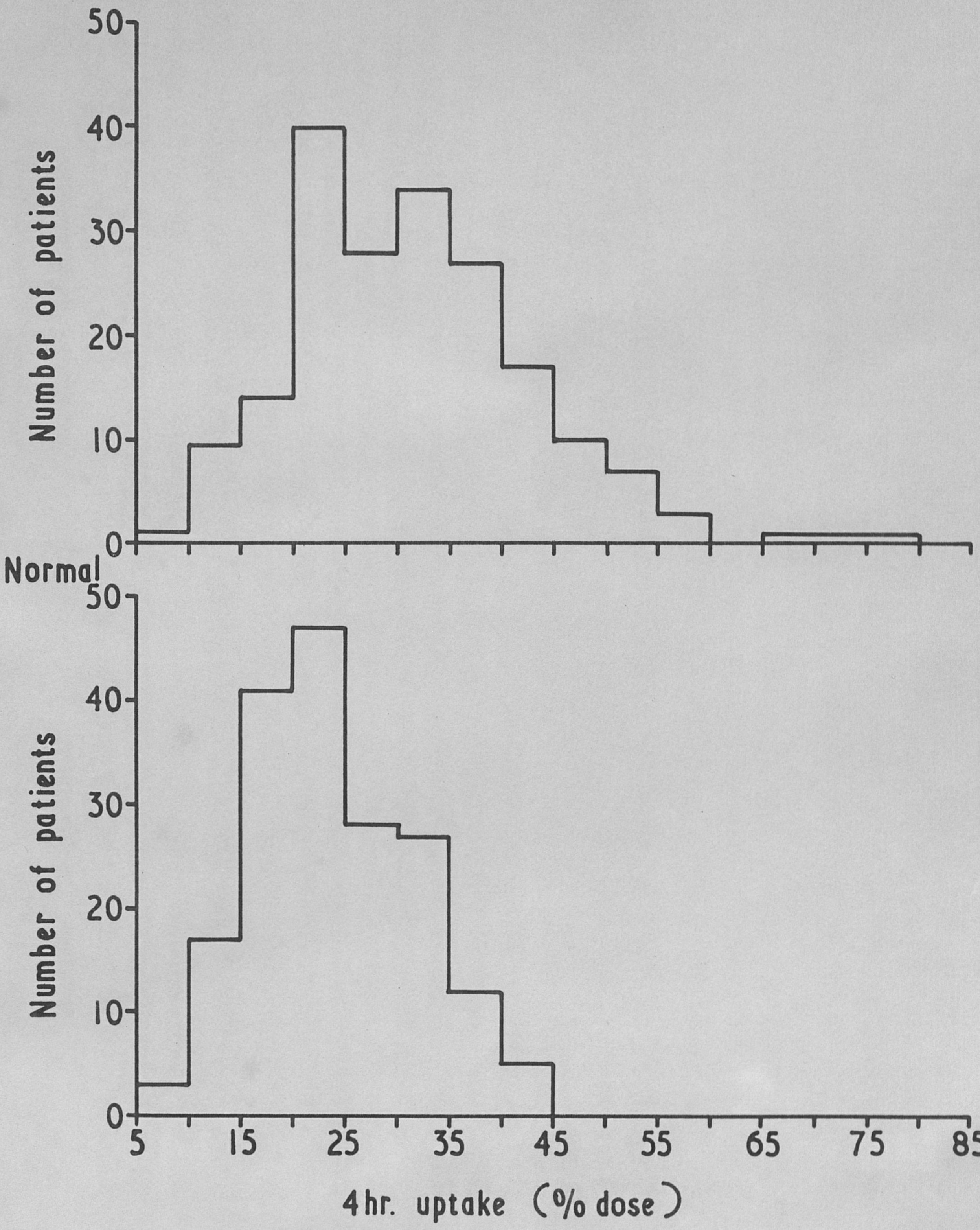

Fig. 25. Distribution of 4 hr. uptakes of ^{131}I of 194 patients with simple goitre, and of 180 patients without thyroid enlargement.

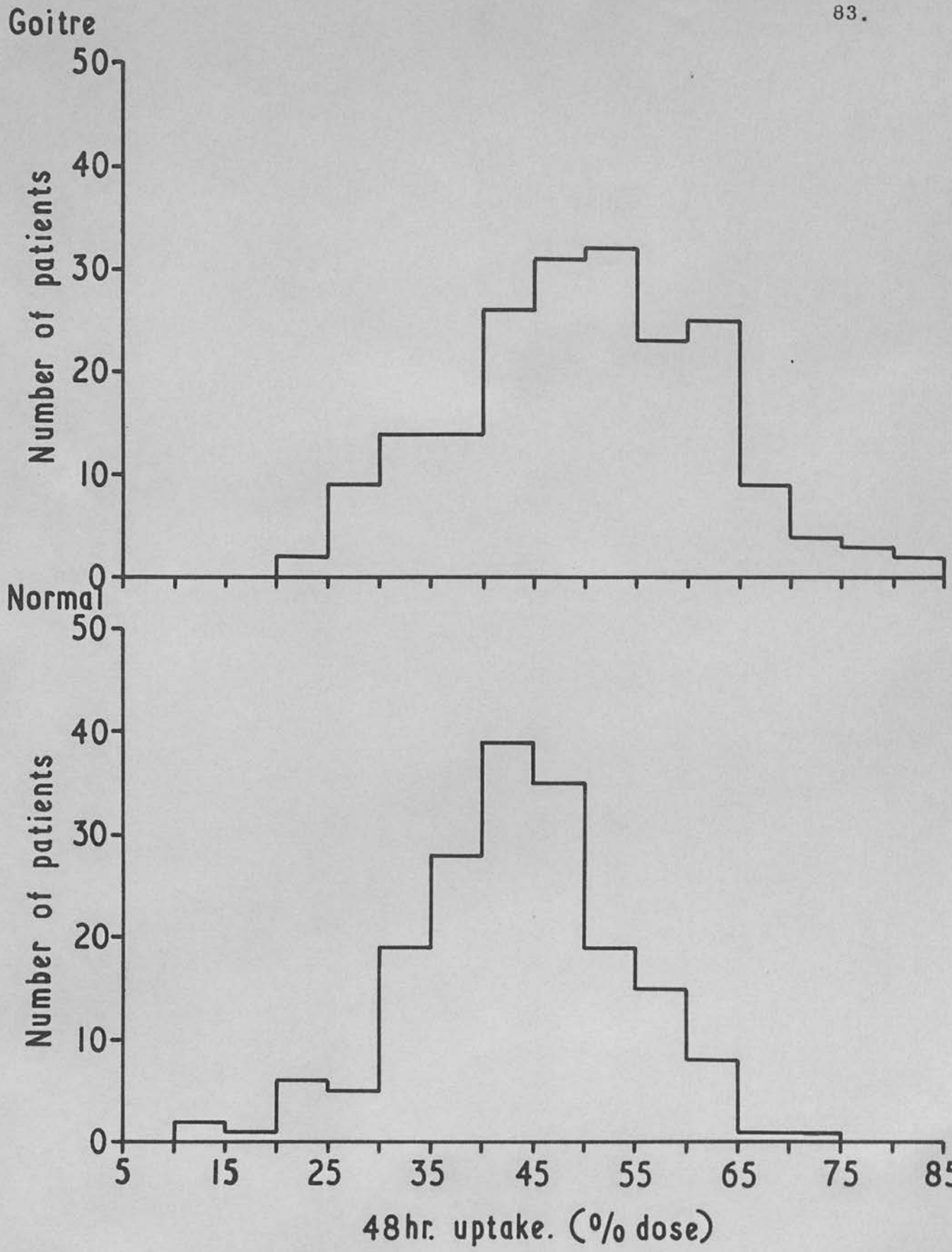

Fig. 26. Distribution of 48 hr. uptakes of ^{131}I of 194 patients with simple goitre, and of 180 patients without thyroid enlargement.

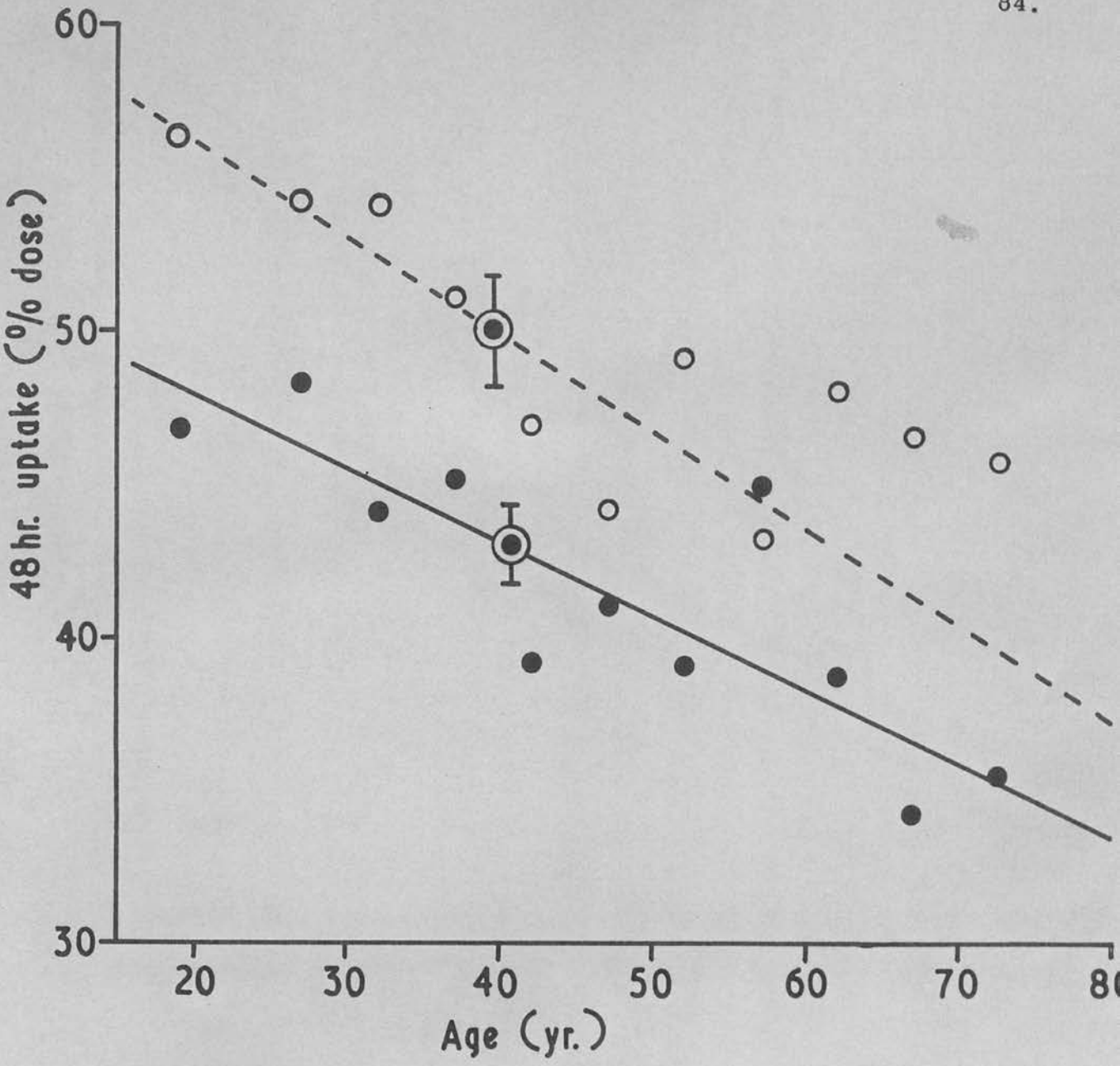

Fig. 27. Effect of age on 48 hr. uptake of ^{131}I in patients with simple goitre (open circles) and patients without thyroid enlargement (closed circles). The values given are the means for half decades except that left hand values are for ages up to 25 yr. and right hand values for ages over 70 yr. The linear regression lines of uptake on age are indicated. The means for all patients in each group are given as double circles, and standard errors of these means are shown as vertical lines.

Age group	Simple Goitre					Normal			
	No. in each group	Mean thyroid uptake at 4 hr. (% dose) ± s.e.	Mean thyroid uptake at 48 hr. (% dose) ± s.e.	No. in each group	Mean serum protein-bound ^{131}I concentration at 48 hr. (% dose / litre) ± s.e.	No. in each group	Mean thyroid uptake at 4 hr. (% dose) ± s.e.	Mean thyroid uptake at 48 hr. (% dose) ± s.e.	No. in each group
0 - 24	17	35·6 ± 2·8	56·4 ± 2·4	18	0·083±0·02	14	26·3 ± 2·1	46·7 ± 2·6	9
25-29	28	37·3 ± 2·7	54·3 ± 2·0	21	0·083±0·01	18	26·6 ± 1·7	48·3 ± 1·8	12
30-34	28	34·8 ± 2·5	54·3 ± 2·3	23	0·079±0·03	39	24·8 ± 1·2	44·4 ± 1·6	21
35-39	30	32·2 ± 1·6	51·4 ± 1·9	28	0·123±0·04	30	25·4 ± 1·3	45·4 ± 1·6	12
40-44	31	29·7 ± 1·9	47·1 ± 2·3	29	0·128±0·02	17	22·9m± 2·1	39·1 ± 1·9	12
45-49	23	26·5 ± 2·0	44·2 ± 2·1	21	0·104±0·04	23	22·8 ± 1·7	41·2 ± 1·9	14
50-54	11	29·0 ± 3·0	49·0 ± 3·1	10	0·160±0·04	14	21·5 ± 2·0	39·2 ± 3·4	10
55·59	14	26·1 ± 2·5	43·3 ± 2·8	16	0·168±0·04	9	23·8 ± 2·9	45·2 ± 4·8	4
60-64	4	28·2 ± 4·6	48·4 ± 5·1	5	0·061±0·08	3	19·6 ± 2·2	38·8 ± 5·6	2
65-70	4	30·2 ± 8·3	46·8 ± 8·8	5	0·112±0·09	9	17·6 ± 1·3	34·4 ± 2·6	4
70	4	23·0 ± 8·5	42·1 ± 9·3	4	0·090±0·09	4	19·1 ± 2·6	35·7 ± 5·1	5
Total	194	31·6 ± 0·65	50·1 ± 0·86	180	0·117±0·01	180	23·6 ± 0·49	43·1 ± 0·76	105

individual regression lines, thus providing good evidence that the regressions for goitre and no goitre were parallel. Analysis for separate and parallel regressions was $F = 37{\cdot}08$ ($P < 0{\cdot}001$), indicating that the two populations have different means but a common slope. The slope of this parallel regression line is $-0{\cdot}28$ (s.e. $0{\cdot}14$), and shows a significant fall in uptake with increasing age in both goitre patients and in normals. There was no significant difference in the age distribution or the mean ages of the two groups. (Mean age for goitre was $39{\cdot}5 + 0{\cdot}87$ and $40{\cdot}3 + 0{\cdot}95$ for normal group). It is clear that uptake of labelled iodine is dependent on age, and that it is higher at all ages in patients with simple goitre. Stanbury *et al.* (324) found a mean 48 hr. uptake of 58·6% in patients with endemic goitre in Mendoza, but age was not considered. The mean age of his group of 101 patients must have been lower than the mean age (40 yrs.) in the present study, as 83% of his patients were less than 40 yr. The mean uptake of the Sheffield patients under 35 yr. is not significantly different from the mean uptake in Mendoza. Without considering effect of age, Skanse (316) reported a mean uptake of 37·5% from a group of 53 normal subjects in Boston, which is significantly lower than the mean uptake of 43·1% for 180 normal subjects from Sheffield. Quimby *et al.* (285) found a correlation of 24 hr. uptake with age, but did not consider it important.

The release of radioiodine by the thyroid gland

Measurements of plasma protein-bound ^{131}I were made at 48 hr. after the dose in 180 patients with goitre and 105 normal subjects. The results are given in Table 13, and are shown in Figs. 28 and 29 plotted against uptake of labelled iodine. There is no correlation with age in either group, as there was with uptake. Mean plasma protein-bound ^{131}I of goitrous patients was 0·117% dose/litre ± s.e. 0·01, and of normal subjects was 0·088% dose/litre ± s.e. 0·01. This difference is not significant, but from Figs. 28 & 29 it is clear that several individual patients with goitre have high values, six being more than 0·4% dose/litre. This level usually indicates hyperthyroidism (370), and is a reflection of the rapid turnover of iodine in this condition. That protein-bound ^{131}I may be above this level in simple goitre has been shown by Wayne (370) and by Burrell and Fraser (50). Its significance will be discussed with the results of total thyroidal iodine measurements (p. 92). No correlation existed between uptake and protein-bound ^{131}I for either goitrous patients or normals. The distributions are shown in Figs. 28 and 29, and correlation coefficients are r = 0·104 for goitre and r = 0·136 for normal groups.

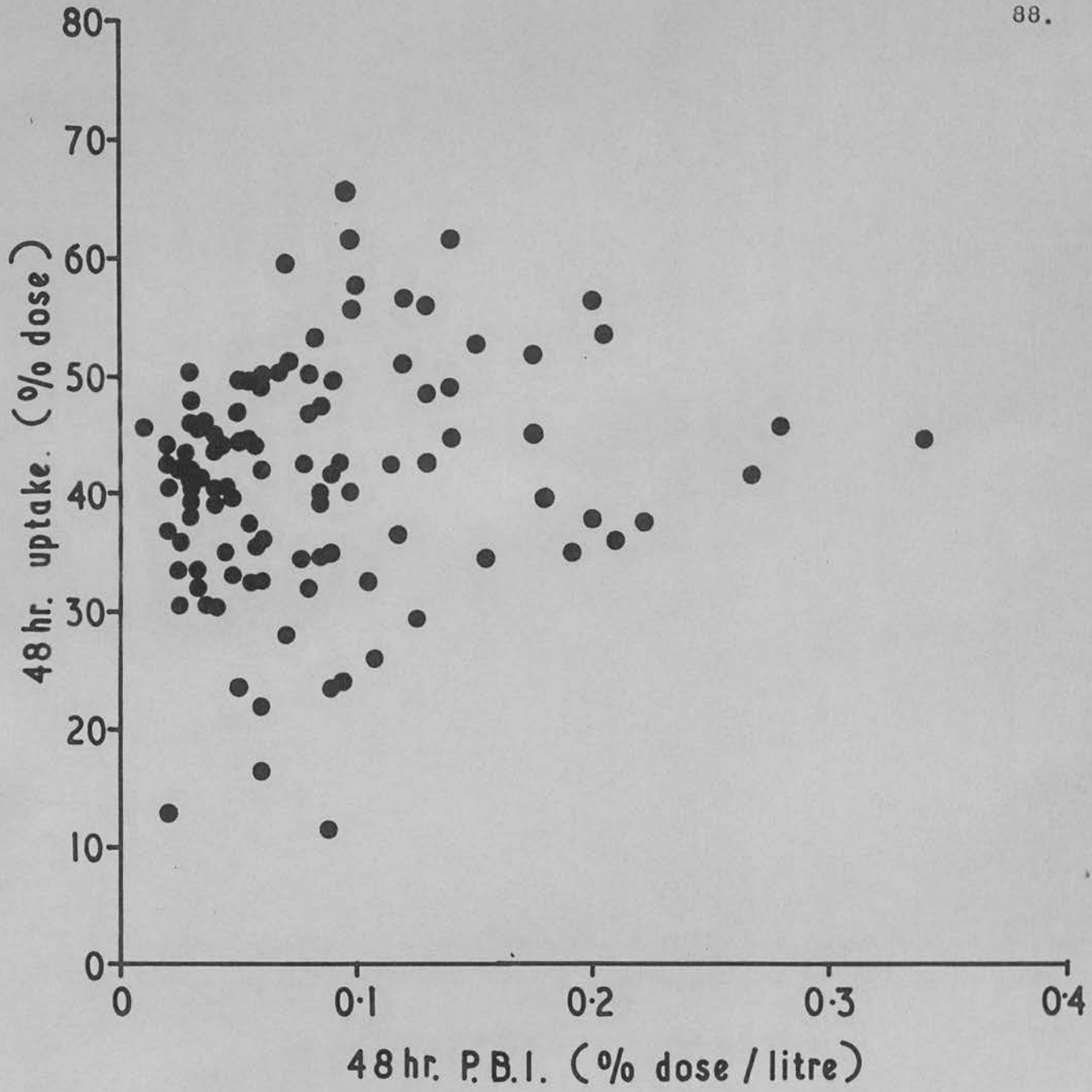

Fig. 28. Uptake of ^{131}I at 48 hr. plotted against plasma protein-bound ^{131}I at 48 hr., in 105 patients without thyroid enlargement.

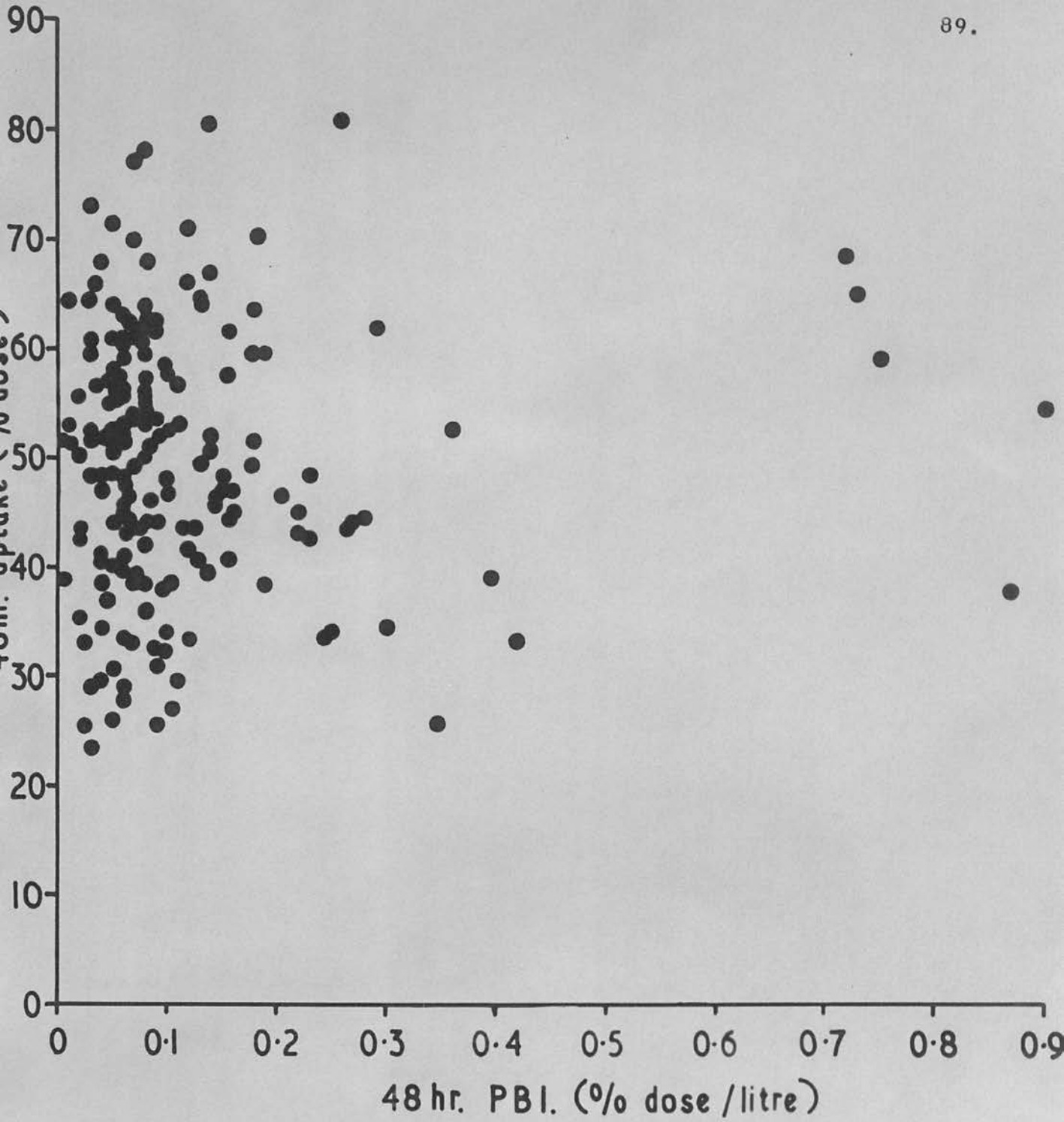

Fig. 29. Uptake of ^{131}I at 48 hr. plotted against plasma protein-bound ^{131}I, in 180 patients with simple goitre.

Serum protein-bound iodine

This was measured in 96 patients with simple goitre and in 95 normal subjects, the groups being of similar age distribution. The results have been grouped in 0·5 µg. intervals and plotted as a frequency histogram in Fig. 30. Clearly the distributions are different, with an increased number of low values in goitre. Mean protein-bound iodine for goitre patients is 5·18 µg./100 ml. ± s.e. 0·11, and 5·81 µg./100 ml. ± s.e. 0·09 for normal subjects, this difference being significant ($P < 0·01$), and the ranges are 2·8 - 7·4 µg. and 4·2 - 7·3 µg. respectively. In Mendoza, Stanbury *et al.* (324) had a wide range of 2·2 - 9·4 µg.% in patients with goitre, but estimations were not done on subjects without goitre from that region. Lamberg *et al.* (186, 187) found that certain of their patients with goitre in Finland had subnormal values for P.B.I., which agrees with the present results in Sheffield. However Querido *et al.* (281) found normal P.B.I. values in the Netherlands, both in patients with goitre (5·6 ± SD. 0·5) and in control subjects (5·7 ± SD. 1·0). Costa *et al.* (80) also found normal values for goitrous subjects in Italy. In Central America Scrimshaw (303) found low values in both goitrous subjects and those without goitre. It would appear that in some areas of the world,

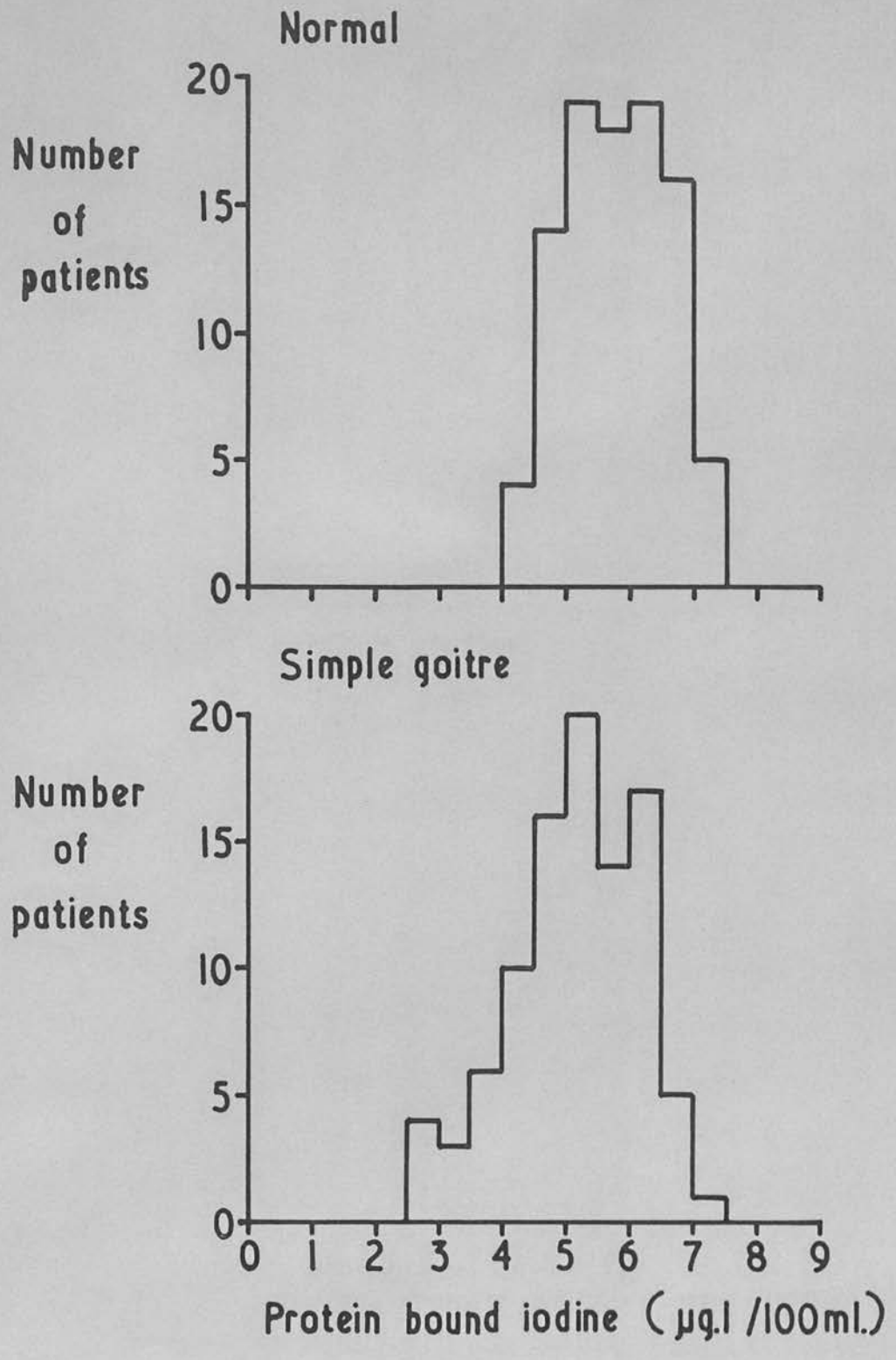

Fig. 30. Distribution of serum protein-bound iodine by 0·5 µg. intervals in 95 normal persons and 96 patients with simple goitre.

simple goitre is associated with low protein-bound iodine values, and that the mean P.B.I. is significantly lower in goitrous patients compared to normal subjects in Sheffield.

There was no correlation between protein-bound iodine and ^{131}I uptake as shown in Figs. 31 and 32, though a few individuals showed very high uptakes with low P.B.I's. Correlation coefficients were -0·12 for 4 hr. and -0·10 for 48 hr. uptakes. Stanbury et al. (324) found that this relationship was significant in Mendoza. In Fig. 33 protein-bound iodine has been plotted against 48 hr. P.B. ^{131}I, and no correlation found ($r = 0·06$).

The iodine content of the thyroid gland

Total iodine content of thyroid glands in simple goitre is of great interest in relationship to the aetiological importance of iodine deficiency. This has been measured in 20 patients with simple goitre and in two patients without thyroid enlargement or endocrine disease, using the method of Nodine et al. (259). A large dose of ^{131}I is required with this method, as accurate measurements of serum radioactivity are made for the 10 days following the dose. The dose given was 200 μc. of ^{131}I, and this dose delivers a significant radiation dose to the thyroid gland and gonads. Therefore all patients were over 40 yr. and the two patients without thyroid enlargement had severe angina pectoris and later received large doses of

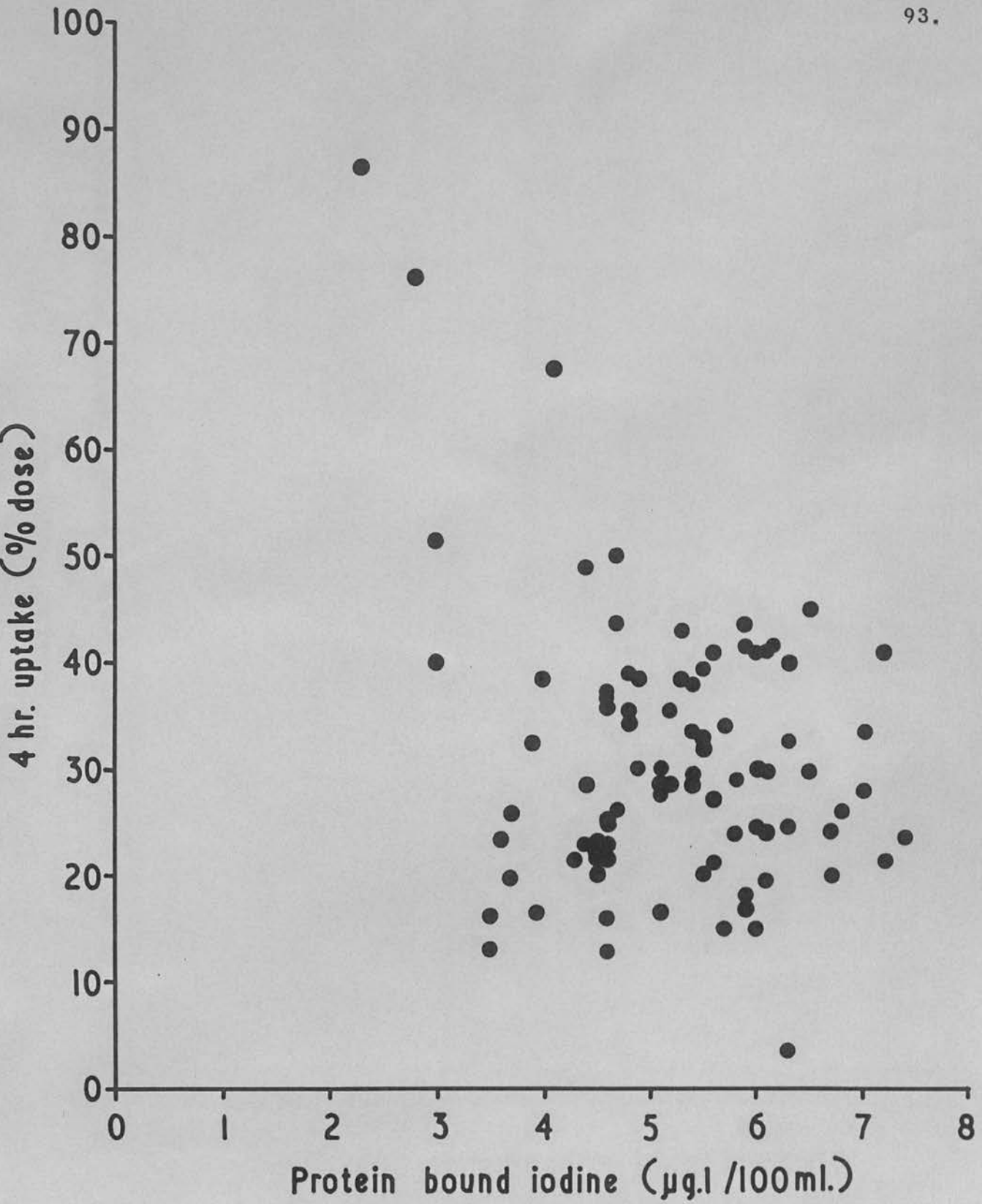

Fig. 31. Uptake of ^{131}I at 4 hr. plotted against serum protein-bound iodine in 96 patients with simple goitre.

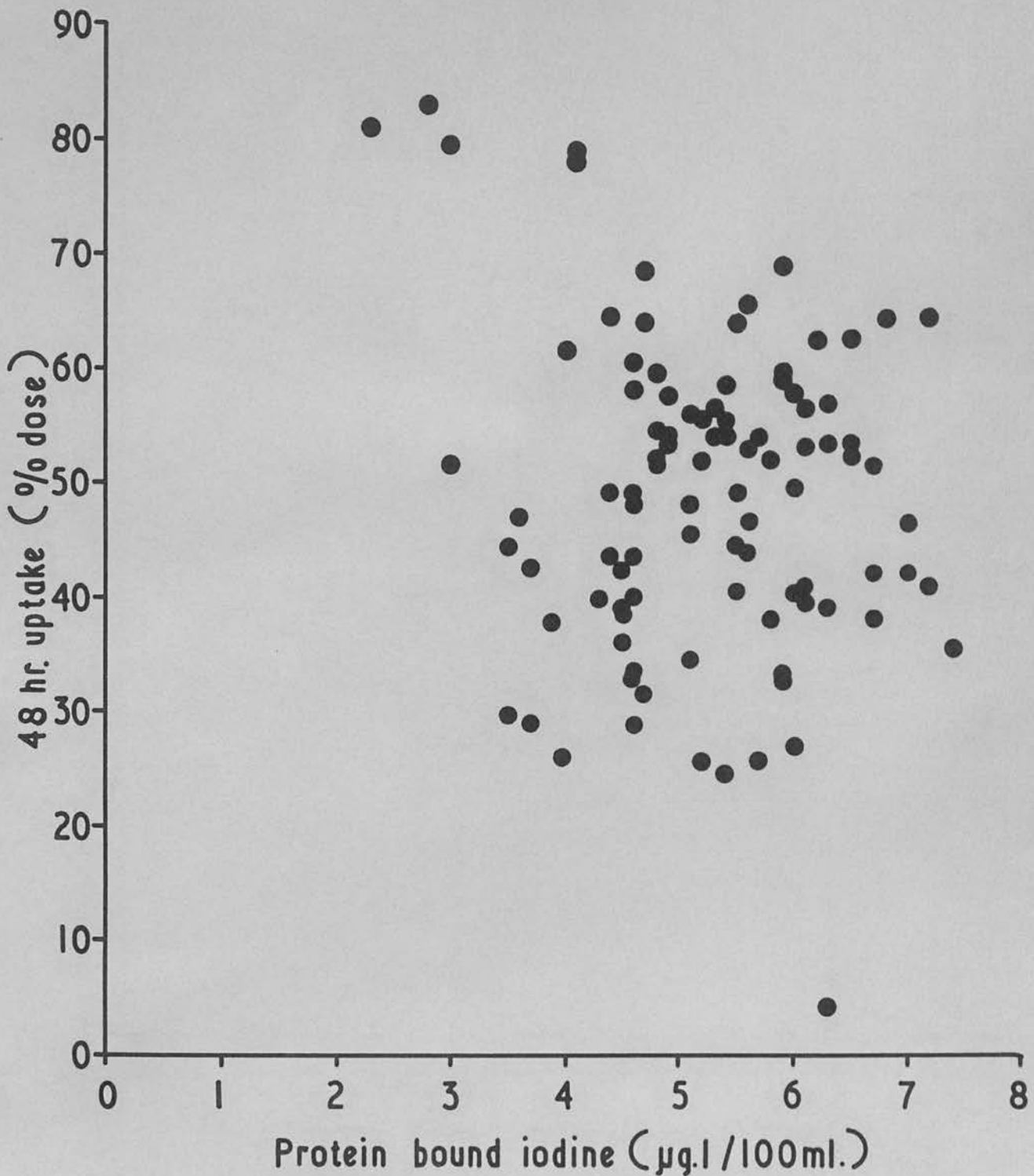

Fig. 32. Uptake of ^{131}I at 48 hr. plotted against serum protein-bound iodine in 96 patients with simple goitre.

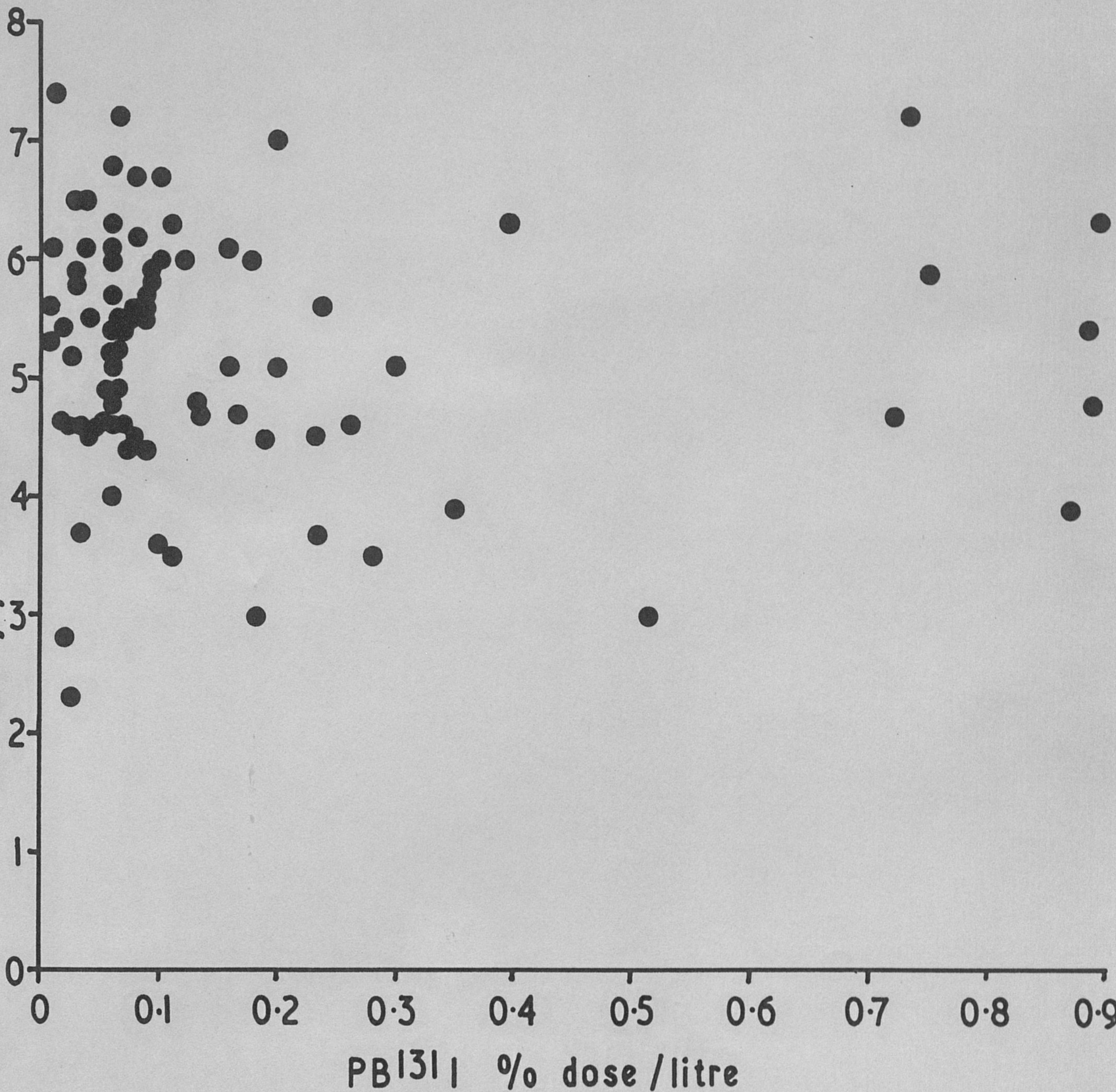

Fig. 33. Serum protein-bound iodine plotted against serum protein-bound ^{131}I in 96 patients with simple goitre.

^{131}I to induce hypothyroidism. Fig.34 shows graphically the measurements which are made, and the complete data required in the calculation of iodine content of the thyroid gland are given in Table 14 . An illustrative calculation is described in detail in Appendix 4.

This method measures the amount of iodine in the thyroid gland which exchanges with ^{131}I and is therefore being used in hormone synthesis and release. It is entirely possible for this amount to be smaller than the total content of iodine in the thyroid, if there is any non-exchangeable iodine within the gland. The latter is also measured, when chemical estimation is performed on the excised gland. This possibility and the accuracy of the method were assessed by measuring the iodine store immediately before partial thyroidectomy in 8 patients with simple goitre, and the results are shown in Table 15 . The operation was performed on the first day after the injection of thyrotrophin, and immediately following the taking of blood for specific activity measurements. Two or three pieces of thyroid tissue were taken from the excised gland, and stable and labelled iodine were measured. If the method is accurate and there is no non-exchangeable thyroid iodine, the specific activity of the iodine released from the gland by thyro-

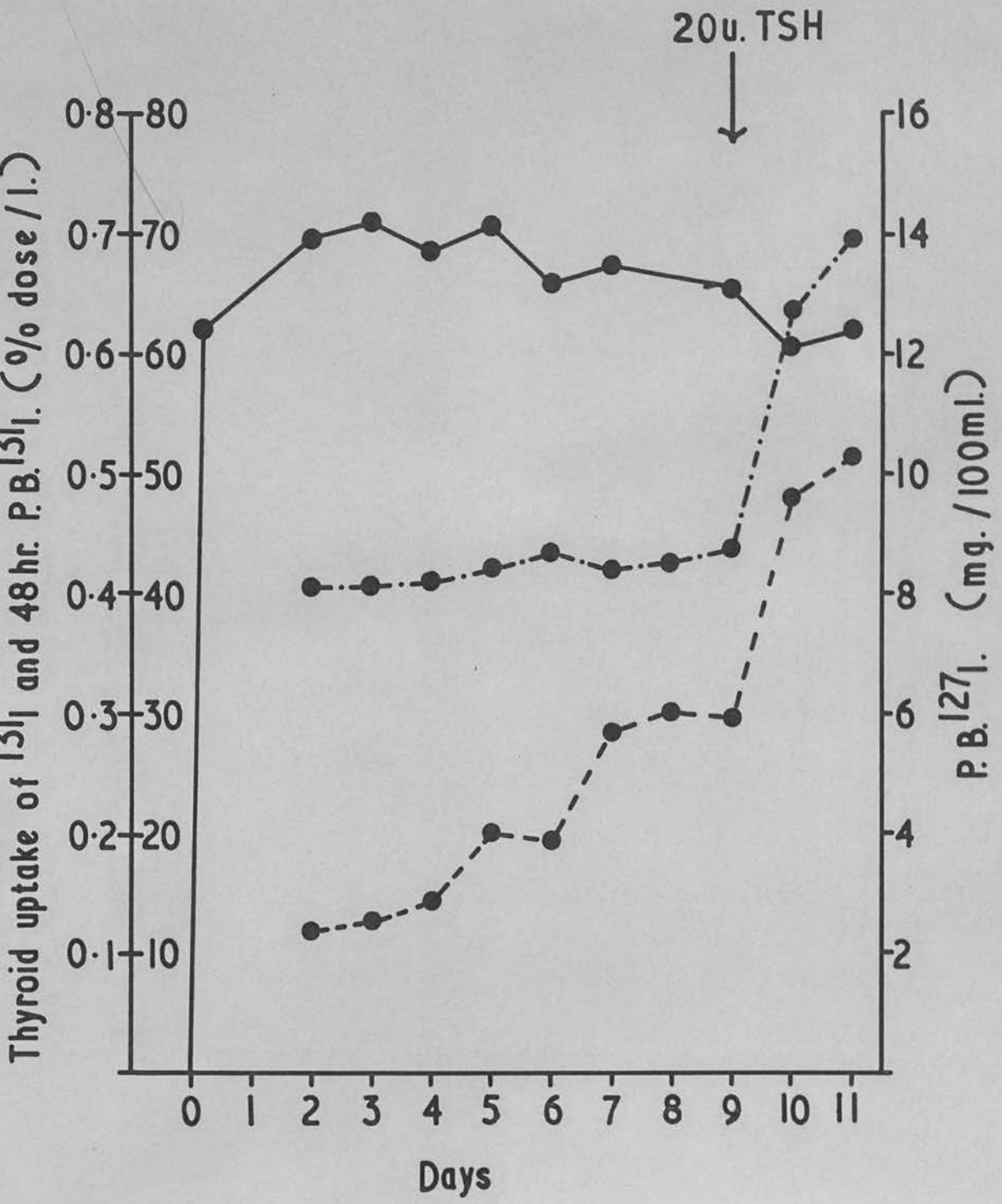

Fig. 34. Measurements of uptake of ^{131}I, serum protein-bound ^{131}I and serum protein-bound iodine made in calculating thyroid total iodine content.
Female D.L. 46 yr. simple goitre for over 10 yr.

Table 14. Measurements of protein-boun
content of ^{131}I before and after 2
thyroidal content of ^{127}I, in 20

Case	Sex	Mean PB^{127}I before T.S.H. µg./100 ml.	Mean PB^{131}I before T.S.H. % dose/ litre	Increase in PB^{127}I on day after T.S.H. µg./100 ml.	Increas in PB^{13} on day after T % dose/
A.L.	M	6·32	0·206	3·44	0·16
B.L.	F	6·10	0·512	1·81	0·22
M.S.	F	7·00	0·259	1·79	0·08
A.B.	F	6·75	1·142	1·11	0·21
J.H.	F	4·51	0·091	1·40	0·04
J.H.	F	4·83	0·260	3·05	0·13
J.C.	M	4·61	0·104	2·53	0·08
V.D.	F	4·84	1·256	0·64	0·11
A.L.	F	5·91	0·679	2·66	0·34
I.S.	F	6·99	0·267	2·06	0·07
W.F.	F	6·82	0·120	4·29	0·13
C.A.	M	5·90	1·019	3·10	0·38
B.M.	F	4·37	0·195	3·62	0·28
A.S.	F	6·45	0·126	4·90	0·20
E.C.	F	3·96	1·090	2·88	0·34
C.A.	F	4·54	0·166	3·17	0·21
F.B.	F	8·54	0·297	4·16	0·18
D.L.	F	7·73	0·240	6·56	0·33
L.D.	F	5·31	0·305	2·04	0·27
E.W.	F	7·80	0·411	4·62	0·29
I.W.	F	4·75	0·286	4·95	0·26

Table 15. Thyroid content of iodine, between specific activities and i operation and in removed thyroid

Case	Sex	Diagnosis	Calculated thyroid content of ^{127}I	Specific activity of thyroid hormone released after T.S.H. % dose ^{131}I/mg. ^{127}I	Mean spec activity thyroid t removed % Dose ^{13} mg. ^{12}
R.T.	M	Normal	5·7	5·58	
J.F.	M	Normal	10·1	4·46	
A.L.	M	Simple Goitre	6·3	4·91	
B.L.	F	"	3·3	12·43	
M.S.	F	"	8·6	4·50	
A.B.	F	"	1·2	19·01	
J.H.	F	"	10·3	3·37	
J.H.	F	"	10·4	4·36	
J.C.	M	"	8·7	3·42	2·93
V.D.	F	"	2·3	18·59	
A.L.	F	"	3·5	13·12	
I.S.	F	"	15·2	3·64	
W.F.	F	"	20·0	3·10	3·17
C.A.	M	"	4·4	12·29	
B.M.	F	"	5·8	7·84	7·57
A.S.	F	"	8·6	4·27	4·09
E.C.	F	"	2·2	11·80	10·94
C.A.	F	"	5·3	6·80	5·83
F.B.	F	"	15·2	4·40	

trophin, and that measured on the excised tissue should be identical. In all but one of the 8 patients there was agreement within 10% and within 20% in the remaining patient. This is strong evidence of the accuracy of the method, and there appears to be an insignificant non-exchangeable iodine compartment in goitrous glands. The total iodine content of the excised gland was always less than the calculated content, this being accounted for by the residual thyroid tissue left by the surgeon.

Thyroidal iodine content range was 1·2 - 20·0 mg. in these 20 patients with goitre. The measurement was repeated in one patient, J.H., nine months later and gave the same result, this being further confirmation of the accuracy of the method. The mean content was 7·7 ± s.e. 1·0 mg., and the results for two normal glands were 5·7 and 10·1 mg. McClendon (206) found a mean of 7·8 mg. for 82 normal glands from two parts of U.S.A. Stanbury et. al.(324) measured iodine content of 17 goitrous glands and found a range of 0·26 - 17·6 mg. and the mean calculated from their values is 6·0 mg. There is thus little change in the mean content of iodine in simple goitre, but some very low values and strikingly some very high values are found, both in Sheffield and in Mendoza. In Table15 the thyroidal concentrations of iodine are given, calculated from the measured iodine content and the clinical estimate

of weight. These are to be compared with the concentrations which were directly measured in the 8 excised glands. In this case, the agreement is only within 50%, though the means are not significantly different. This might be expected from the large errors of clinical estimation of thyroid size. It is clear that all the goitrous glands had a lower concentration of iodine than the two normal glands, and the mean is 0·118 $\pm$ 0·017 mg./g. wet weight. Marine and Lenhart (236) showed that the concentration of goitrous glands was about 1 mg./g. dry weight, compared to the normal gland, which was above 2 mg./g. dry weight. The mean water content of the excised glands in Sheffield was 74%, and if this value is used to correct the above mean concentration, it then becomes 0·472 mg./g. dry weight. Stanbury *et al.* (324) does not give calculated concentrations of iodine in the goitrous glands, but as most of the patients investigated had glands between 50 and 200 g., it is clear that the iodine concentration in Mendoza was similar to that found in Sheffield, and that both are much lower than the iodine concentration of normal glands.

Total iodine content of these 20 goitrous glands is plotted against various other parameters of thyroid function in Figs. 35, 36, 37. A logarithmic conversion of thyroid iodine gives a more normal distribution against 48 hr. uptake of ^{131}I, and the

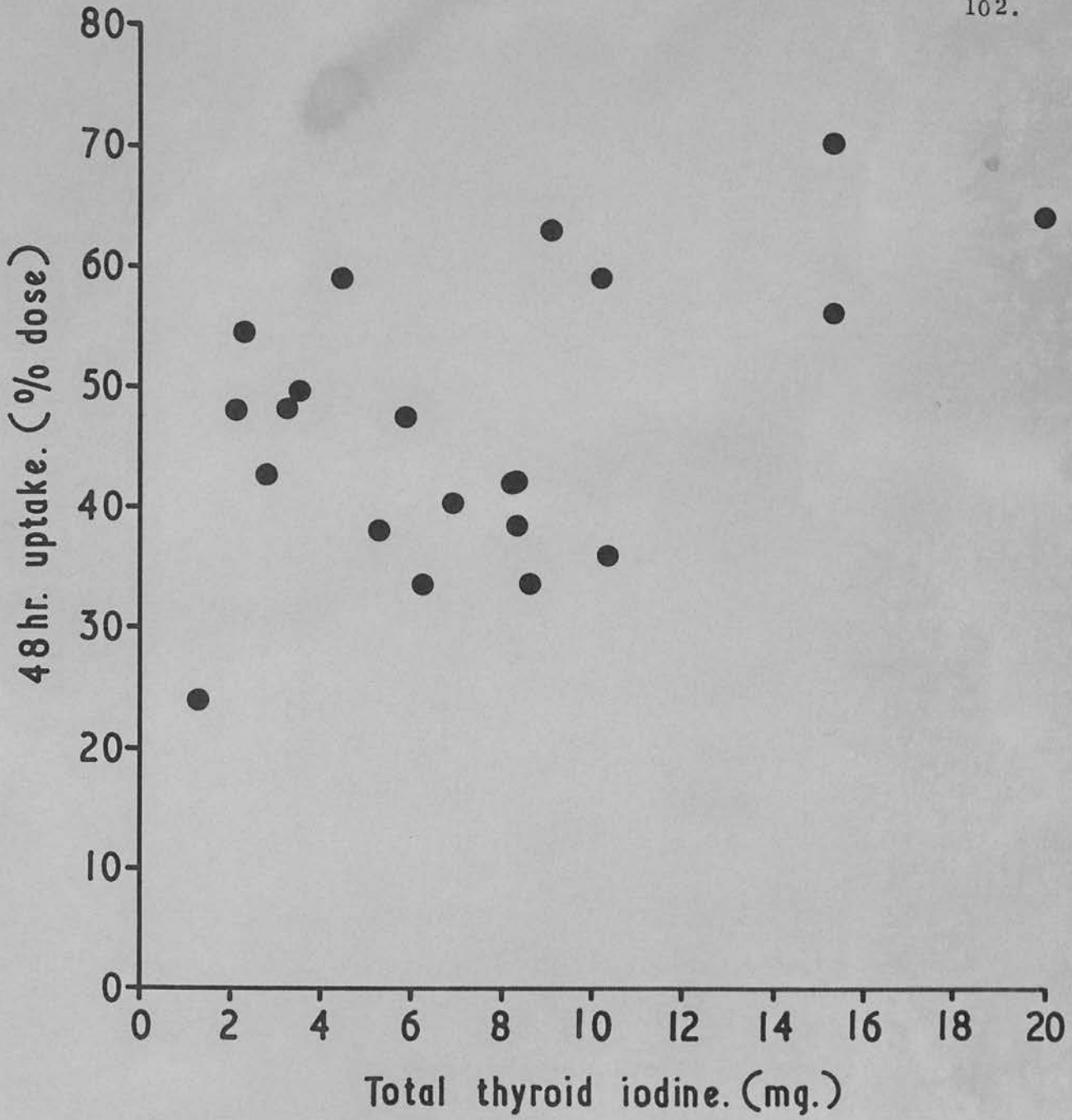

Fig. 35. Relationship between uptake of ^{131}I at 48 hrs. and calculated thyroid iodine content in 20 patients with simple goitre. Logarithmic conversion of iodine content gives a significant correlation $r = 0{\cdot}498$, $P < 0{\cdot}05$.

correlation coefficient r = 0·498 ($P < 0·05$) is significant. It is striking that the correlation is positive, so that uptake increases with increasing thyroid iodine content. The latter is shown against 48 hr. P.B ^{131}I in Fig. 36 . This distribution is not normal and can only be made so by logarithmic conversion and omitting the four upper points, where r = -0·436 ($P < 0·01$). This is only on the borderline of significance. There is almost complete separation of the P.B ^{131}I values for thyroid iodine contents above and below 5 mg. Low total thyroid iodine is thus associated with P.B ^{131}I values higher than 0·2% dose/litre and from Table 15 , with high specific activity values for hormone released in response to thyrotrophin. A low thyroid iodine content is a reasonable explanation of the occasional high values for P B ^{131}I found in some cases of simple goitre. There is no correlation found between thyroid iodine content and stable protein-bound iodine as shown in Fig. 37 as r = 0·304 ($P < 0·2$). If increased secretion of thyrotrophin from the hypophysis is the mechanism for the increase in thyroid size, it is reasonable to expect blood hormonal iodine concentration to be lower in goitre, thus providing the stimulus to the hypophysis. Evidence that mean serum protein-bound iodine is lower in simple goitre than in patients without thyroid enlargement has already been given.

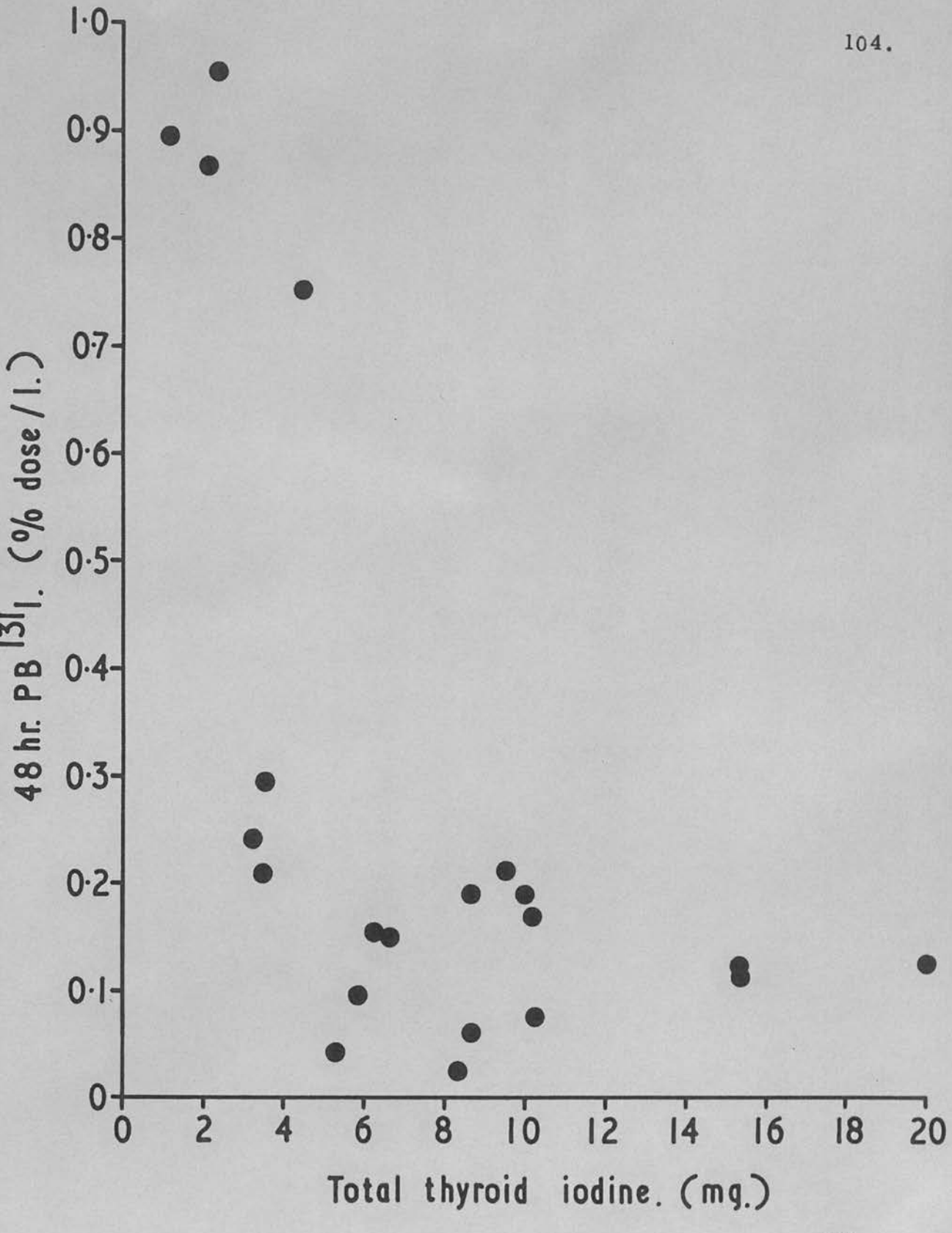

Fig. 36. Relationship between serum protein-bound ^{131}I at 48 hrs. and calculated thyroid iodine content in 20 patients with simple goitre. Omitting the four upper points and logarithmic conversion of iodine content gives a correlation coefficient $r = -0{\cdot}436$, $P < 0{\cdot}10$.

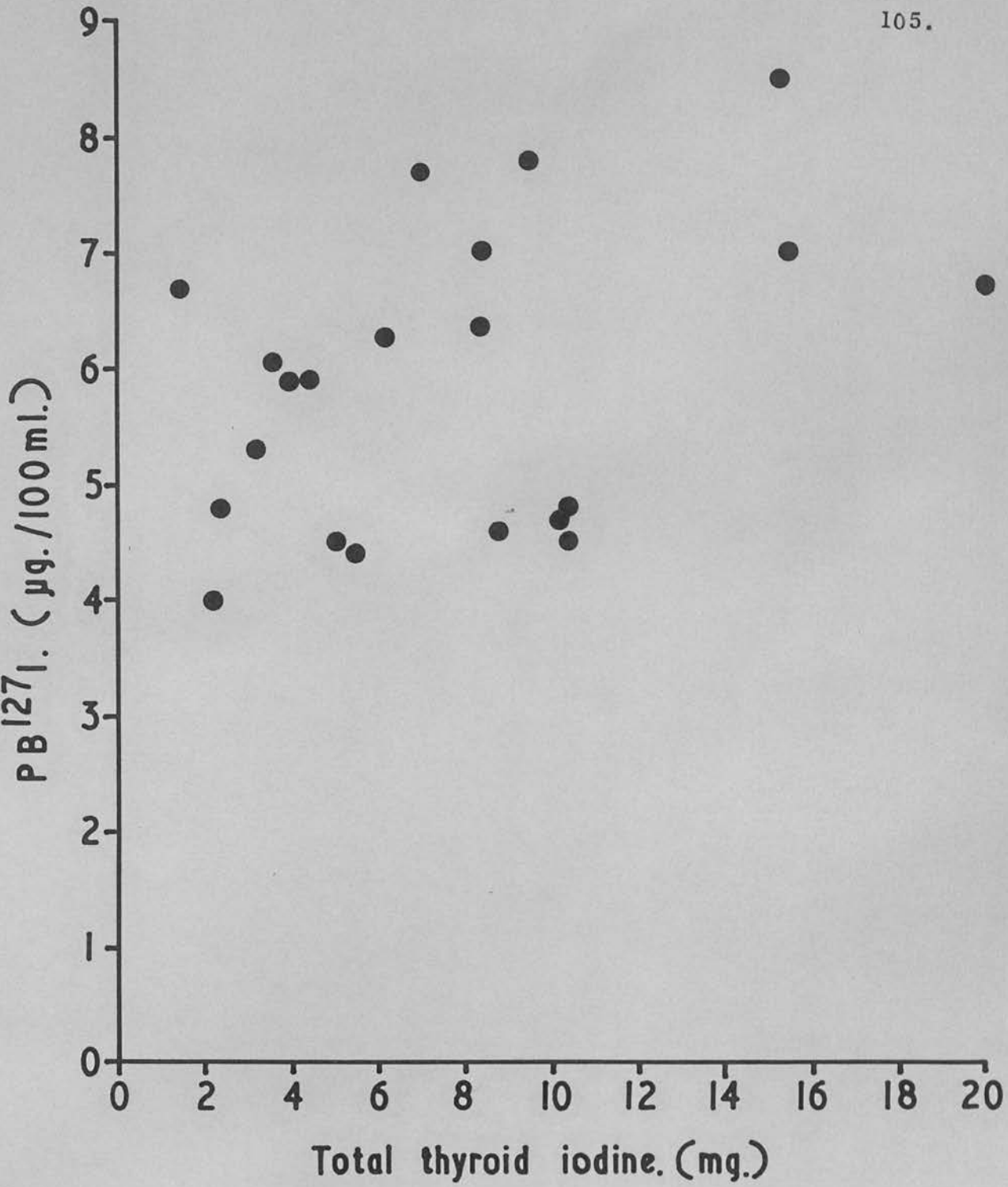

Fig. 37. Relationship between serum protein-bound iodine and calculated thyroid iodine content in 20 patients with simple goitre. There is no significant correlation r = 0·304.

Riggs (292) has shown that the serum level of thyroxine is a direct measure of thyroid hormone synthesis. Thus total iodine content of thyroid appears not to correlate with output of thyroid hormone. Similarly, this output is not related to concentration of thyroid iodine, as there is no significant correlation between serum stable protein-bound iodine and the calculated thyroid iodine concentration as shown in Fig. 38, $r = 0{\cdot}214$ ($P < 0{\cdot}4$).

Thyroid iodine content is significantly correlated with estimated thyroid size as shown in Fig. 39. Converting gland size logarithmically gives a normal distribution and $r = 0{\cdot}525$ ($P < 0{\cdot}02$). Thus total thyroid iodine increases with thyroid size. Therefore the total iodine content of the gland is probably not a stimulus to increase in thyroid size. A striking correlation occurs between concentration of thyroid iodine and estimated thyroid size, and is shown in Fig. 40. A double logarithmic conversion is required to give a normal distribution, when $r = -0{\cdot}454$ and P ($< 0{\cdot}05$.) Clearly thyroid iodine concentration falls with increasing mass of the gland, and could hypothetically be the stimulus for this increase in mass. It may be tentatively suggested that this relationship is not mediated entirely through lowered blood hormonal level and increased hypophyseal secretion of thyrotrophin

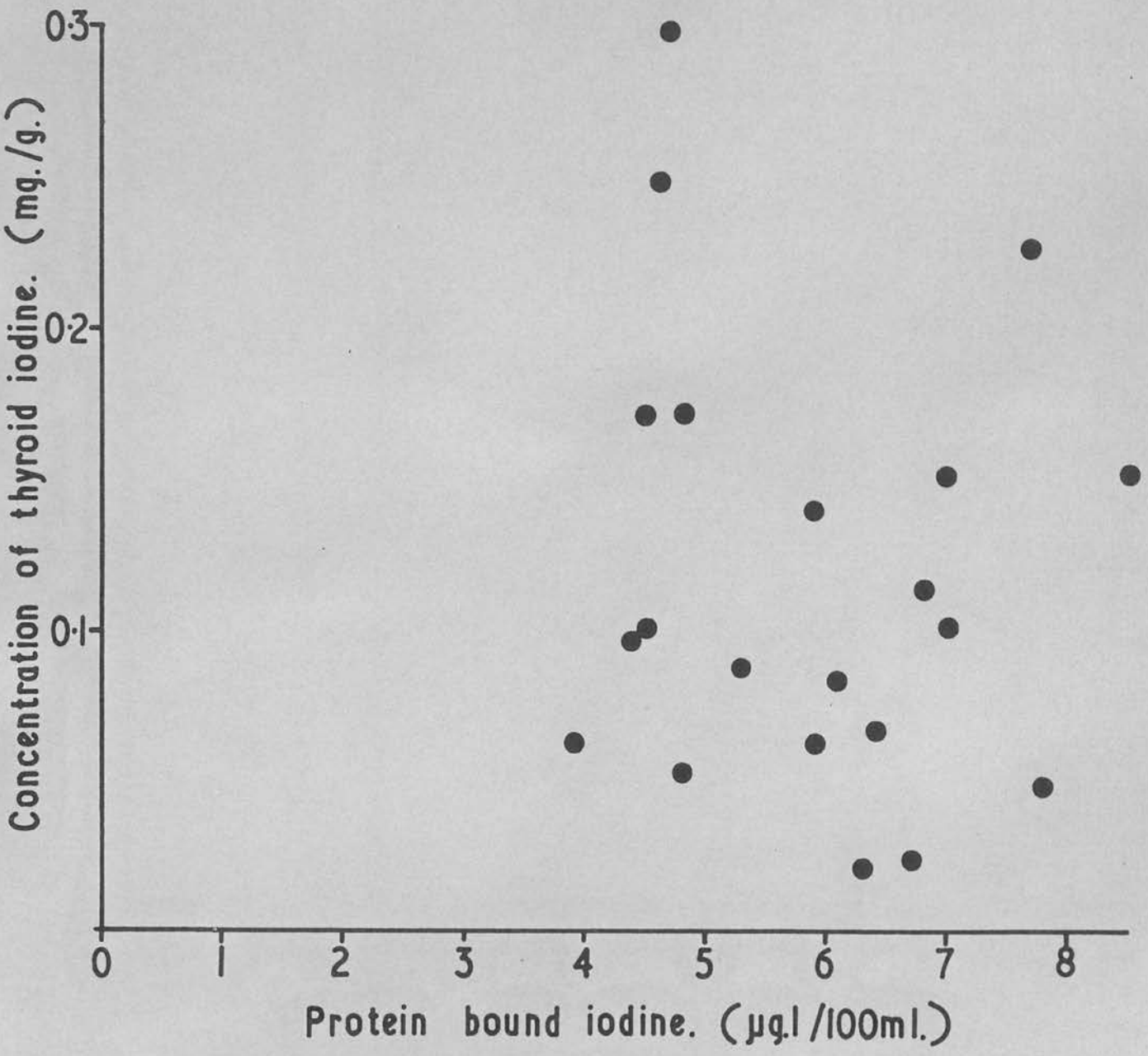

Fig. 38. Relationship between serum protein-bound iodine and concentration of iodine in the thyroid, calculated from the content of thyroid iodine and estimated size of the gland, in 20 patients with simple goitre. There is no significant correlation
$r = 0 \cdot 304$, $P < 0 \cdot 20$.

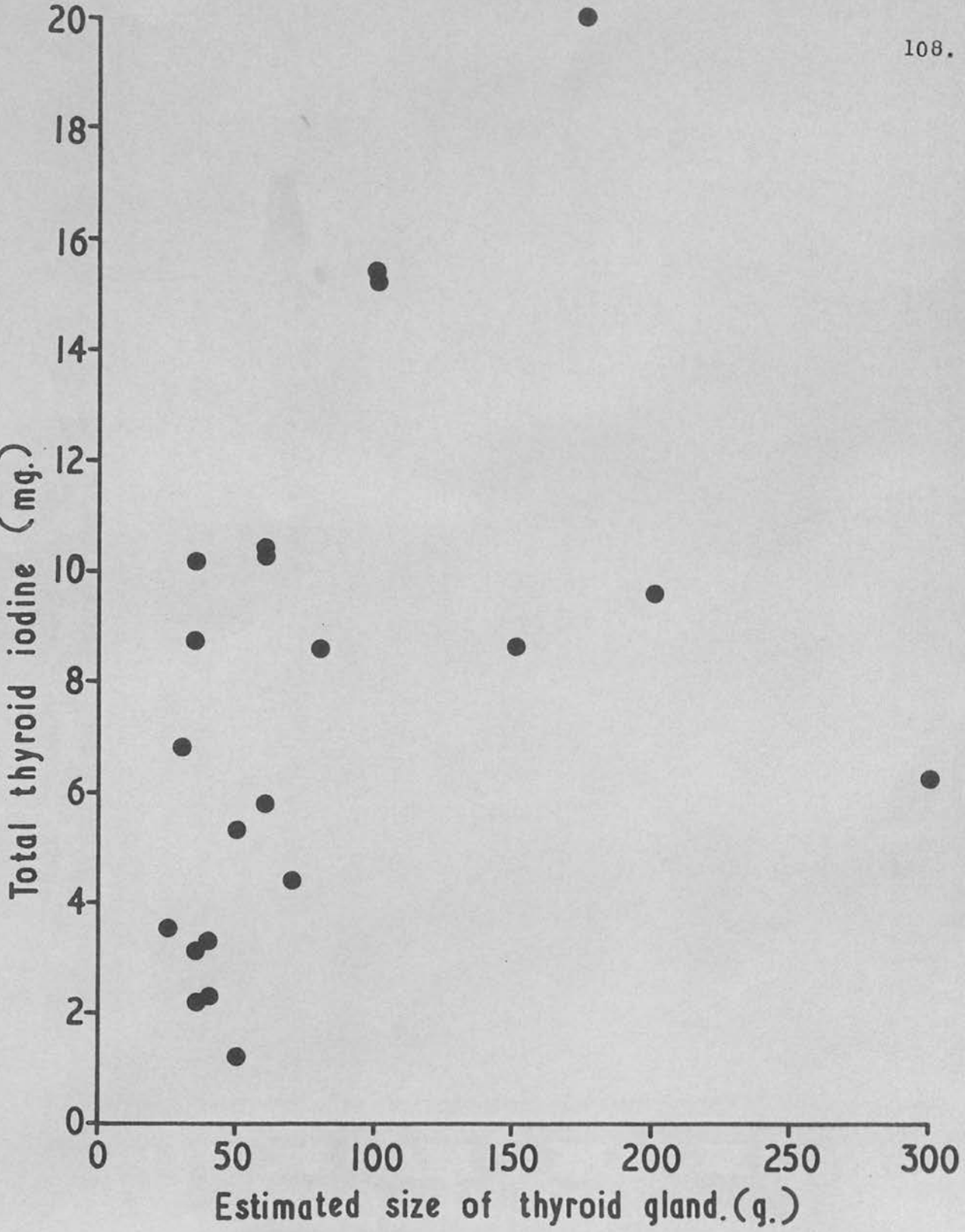

Fig. 39. Relationship between content of thyroid iodine and estimated size of the thyroid in 20 patients with simple goitre. Logarithmic conversion of estimated size gives a more normal distribution and a significant correlation, $r = 0{\cdot}525$, $P < 0{\cdot}02$.

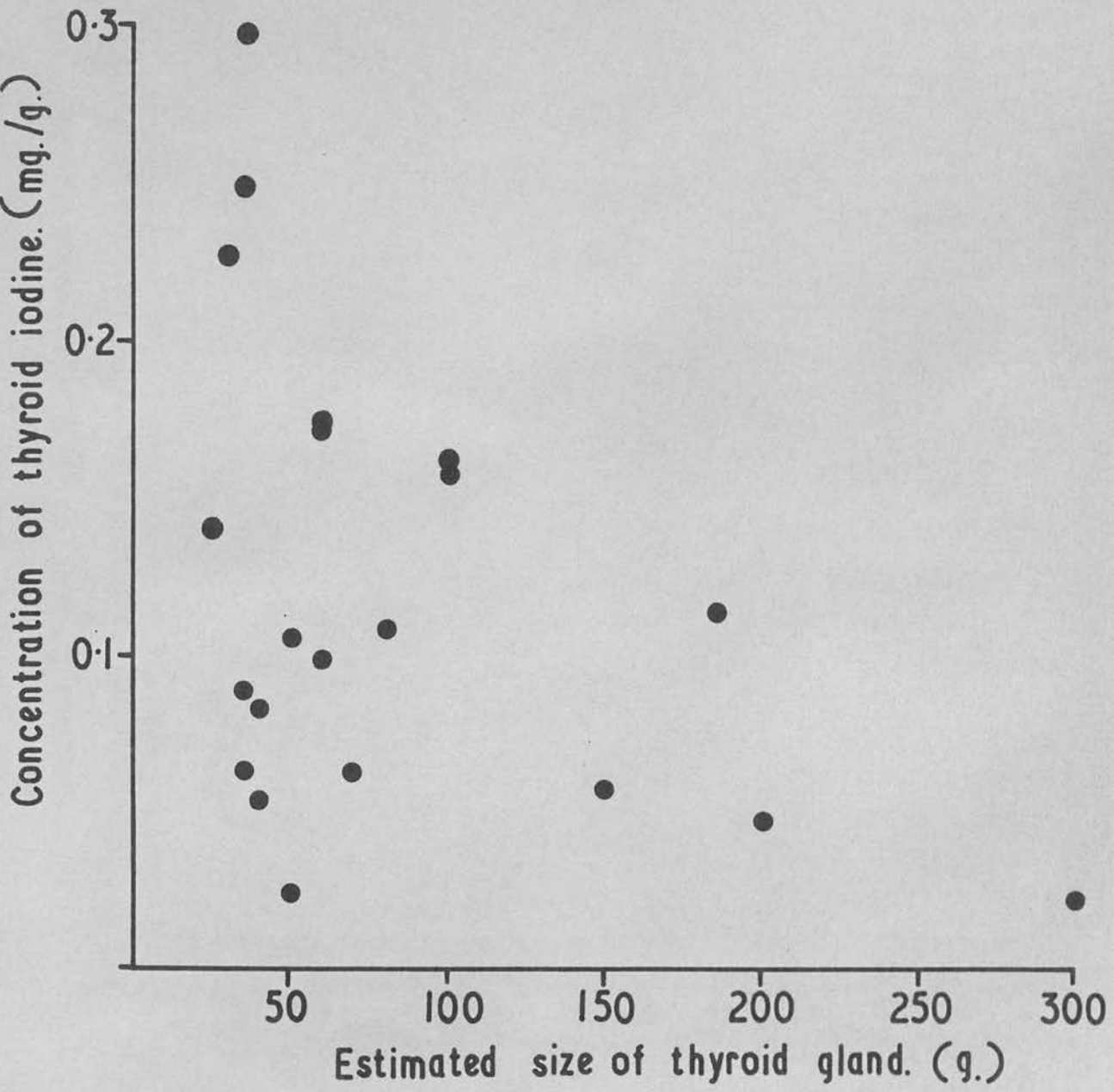

Fig. 40. Relationship between calculated concentration of iodine in the thyroid gland and estimated size of the thyroid in 20 patients with simple goitre. Normal distribution is achieved by double logarithmic conversion and the correlation is significant $r = 0{\cdot}454$, $P < 0{\cdot}05$.

because of the lack of correlation between serum stable iodine and thyroid iodine. Thus the stimulus to increasing thyroid size might be due directly to decreased concentration of thyroid iodine, and this stimulus would be added to that from increased thyrotrophin production due to decreased hormone synthesis. Astwood (13) has already raised this suggestion and there is supporting experimental evidence from Halmi (137), Vanderlann and Caplan (349) and Wahlberg (363).

Biological half-life of ^{131}I in thyroid

This was calculated in 22 patients with simple goitre, by measuring thyroid content of ^{131}I at 48 hr. and 7 and 14 days after a dose of 30 μc. The best possible line to fit these three points was found by least squares and the half-time of ^{131}I in the thyroid measured from this line. The error of this procedure is large for long half-lives, as is seen from the results in Table 16. The mean half-time of thyroidal ^{131}I is 258 ± s.e. 42·7 dy. In normal subjects this measurement varies from 56 dy. to infinity (Quimby, 283). This result suggests that half-life in simple goitre is normal or longer than normal. Certainly it excludes a shorter half-life than normal, which is the expected result when there is increased loss of either inorganic or organic iodine from the body. A shorter half-life would be expected if increased renal or faecal

Table 16. Serum protein-bound ^{127}I concentration, of ^{131}I in serum at 48 hrs. after dose, thyroid renal plasma clearance of ^{131}I in 22 patients w

Case	Sex	Protein-bound ^{127}I concentration (µg./100 ml.)	Thyroidal uptake of ^{131}I at 48 hr. (% dose)	Protein-bound ^{131}I concentration at 48 hr. (% dose)	Thy hal of (da
S.H.	F	5·2	55·7	0·059	3
R.B.	F	5·4	58·5	0·060	3
M.H.	F	6·7			4
J.B.	F	6·3	41·2	0·032	18
M.J.	F	3·5	44·5	0·280	3
E.W.	F	7·1	44·3	0·096	45
S.C.	F	5·7	54·1	0·056	32
J.M.	F	4·0	42·0	0·080	27
A.W.	F	6·3	56·9	0·110	56
E.D.	F	4·6	49·1	0·02	42
E.A.	F	5·2	38·0	0·099	38
E.H.	F	5·4	54·1	0·07	17
J.G.	F	3·6	46·8	0·103	52
M.S.	F	5·6	44·2	0·118	4
J.D.	F	6·8	28·4	0·062	31
D.G.	F	6·5	62·5	0·080	2
D.S.G.	F	5·1	65·2	0·125	3
S.C.	F	5·8	52·0	0·087	3
J.W.	F	7·4	35·3	0·016	46
N.C.	F	6·2	64·2	0·089	54
M.F.	M	5·1	43·0	0·126	26
P.R.	F	2·8	83·0	0·026	49

excretion of iodine occurred in simple goitre, as has been suggested by Cassano et al. (60) and by Hydovitz (160). Stanbury et al. (324) similarly found normal or increased half-times of thyroidal ^{131}I in simple goitre in Mendoza. He noted a rapid disappearance rate in the first 24 hr. in several patients followed by a slowing of the disappearance rate from the thyroid, and suggested a two-compartment system within the thyroid in these cases. This was not found in any case studied in Sheffield.

Renal clearance of iodide

The rate of clearance of radioiodine by the kidneys was measured in 15 patients with simple goitre, using the same procedure as Stanbury et al. (324), except that the ^{131}I was injected intravenously. The results are given in Table 16, along with the uptake of ^{131}I and biological half-life of ^{131}I. Mean renal plasma clearance is 29·7 ± s.e. 2·5 ml./min. and range 15·6 - 54·0 ml./min. These results are within the range of clearances found in normal subjects by Berson et al. (32) and by Koutras et al. (181). Stanbury et al. (324) found normal renal clearance of iodide in simple goitre in Mendoza, as did Koutras et al. (181) in simple goitre in Glasgow. Cassano et al. (59,60) have reported high renal plasma clearance in some cases of simple goitre in Italy, especially in patients with high thyroid uptakes of ^{131}I, and have suggested that this high renal clearance is of aetiological importance.

Organic binding of iodine

Potassium perchlorate was given intravenously to 15 patients with simple goitre, 3 hr. after giving ^{131}I, also intravenously. The effect on thyroid uptake is shown in Table 17 and three individual uptake curves, before and after 100 mg. of potassium perchlorate are shown in Fig. 41. There is no striking fall in uptake in any patient and mean percentage change in uptake 30 min. after perchlorate compared to uptake before is +3·3 ± s.e. 0·7%. When organic binding of iodide is depressed from any cause, a rapid discharge of thyroidal ^{131}I occurs following perchlorate or thiocyanate (Vanderhaan and Vanderhaan, 351). Morgans and Trotter (251) reported that such a discharge was found in some cases of lymphocytic thyroiditis, but did not observe it in simple goitre. Binding is impaired in some cases of congenital dyshormonagenesis as demonstrated by McGirr (213). Roche *et al.* (295) described discharge by perchlorate in patients with simple goitre in South America. However there was no rapid discharge, but a gradual fall in uptake over a period of hours. Floyd *et al.* (107) showed that a discharge of ^{131}I by thiocyanate occurred in nodular goitre, where there was a family history of goitre, but no discharge when family history was negative.

Table 17. Effect of perchlorate on thyroid uptake of ^{131}I in 15 patients with simple goitre

Patients	Percentage change from 2½ hr. thyroid uptake of ^{131}I, 15 min. after 100 mg. of potassium perchlorate i-v.	Percentage change from 2½ hr. thyroid uptake of ^{131}I, 30 min. after 100 mg. of potassium perchlorate i-v.
B.W.	+ 2	- 2
N.C.	+ 6	+ 7
E.W.	- 8	+ 2
G.G.	+ 1	+ 1
P.R.	+ 3	+ 5
J.C.	+ 4	+ 6
G.F.	+ 2	+ 4
M.W.	- 2	- 1
V.J.	+ 8	+10
D.G.	- 4	+ 2
M.S.	+ 6	+10
A.V.	- 2	- 5
B.H.	+ 3	+ 5
D.E.	+ 2	+ 2
R.R.	- 1	+ 1
	Mean ± s.e. + 1·34 ± 0·56	Mean ± s.e. + 3·27 ± 0·72

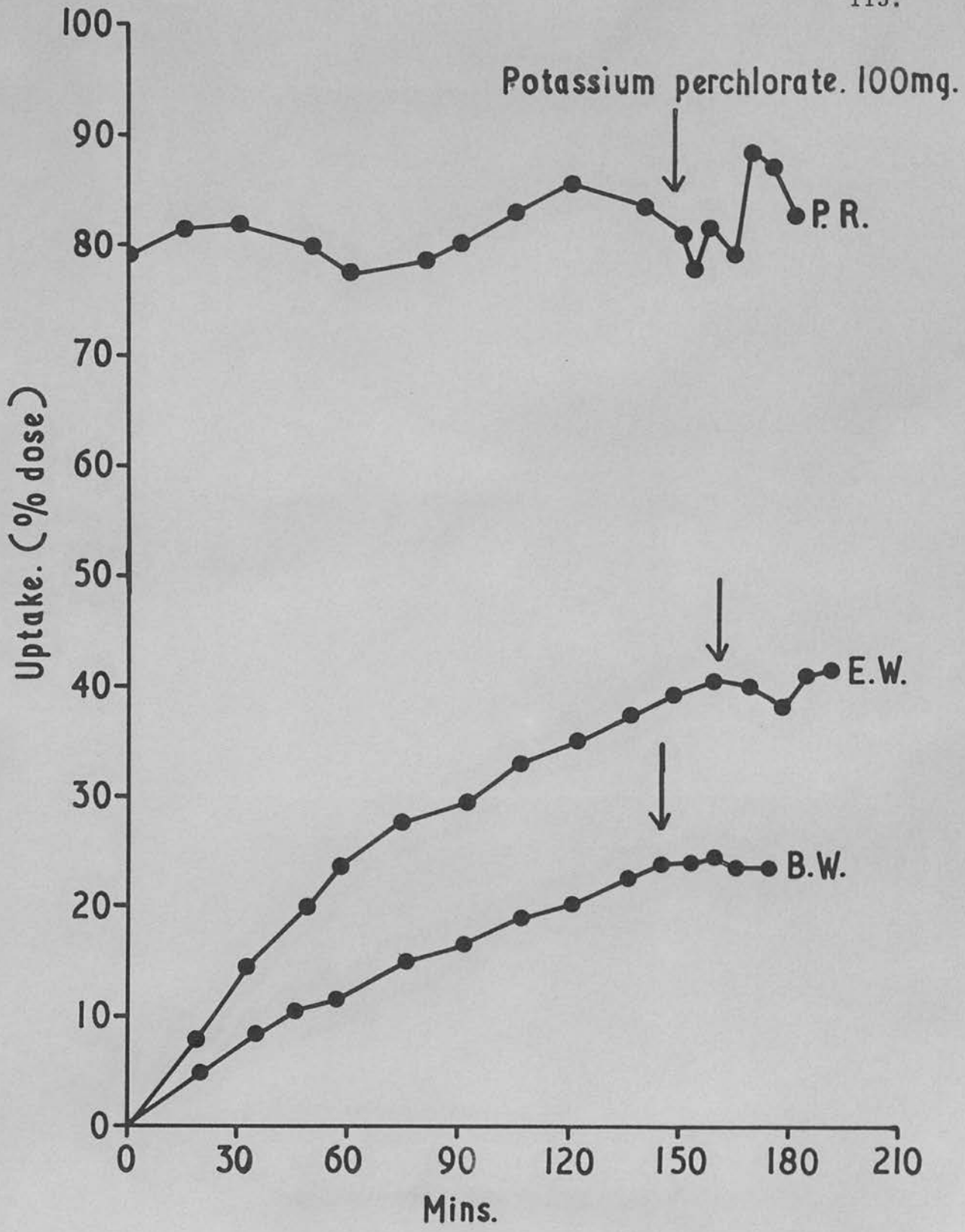

Fig. 41. Three patients with simple goitre given ^{131}I intravenously, and 100 mg. of potassium perchlorate intravenously about 2½ hr. later. No discharge of ^{131}I by perchlorate indicates that all the ^{131}I in the thyroid glands was organically bound.

Morbid histology and autoradiography

Extensive pathological examination has not been carried out in the present study, as the emphasis was on function of the goitrous thyroid gland. Thyroid tissue was obtained in 8 patients who had total thyroid iodine measured, and partial thyroidectomy was also done on several other patients with large simple goitres. Most of these patients had ^{131}I before operation, and autoradiography was done as well as histological examination. All these patients had suffered from goitre for many years and most had large goitres weighing more than 80 g. Histological examination showed the typical features of colloid goitre, often with nodular hyperplasia, and illustrative sections are shown in Figs. 42 - 49 . Aschoff (8) and Wegelin (374) have shown the progressive change in simple goitre from diffuse hyperplasia , to the stage of colloid goitre, where the gland becomes well filled with colloid and very large follicles appear. The final stage is the appearance of nodules and progressive isolation of these nodules as they progressively grow. Distinct capsulation may occur with central degeneration and cyst formation in follicles. All of these features are found in these goitrous glands from Sheffield. Uehlinger (347) and De Smet (88) have recently reported extensive pathological examination of endemic goitre in Switzerland and Belgian Congo.

Autoradiographs from three of these colloid goitres are shown in Figs.50,51.Characteristically, they show concentrations of ^{131}I in small or normal sized follicles and very little appears in macrofollicles. This might be simply due to dilution effect by the much larger amount of colloid in these large follicles, but Fitzgerald (104) reported fully on autoradiography of nodular goitre and showed that this lack of concentration occurred whether the lining cells were large or small. Taylor (335) also showed this localisation of ^{131}I in micro-follicles.

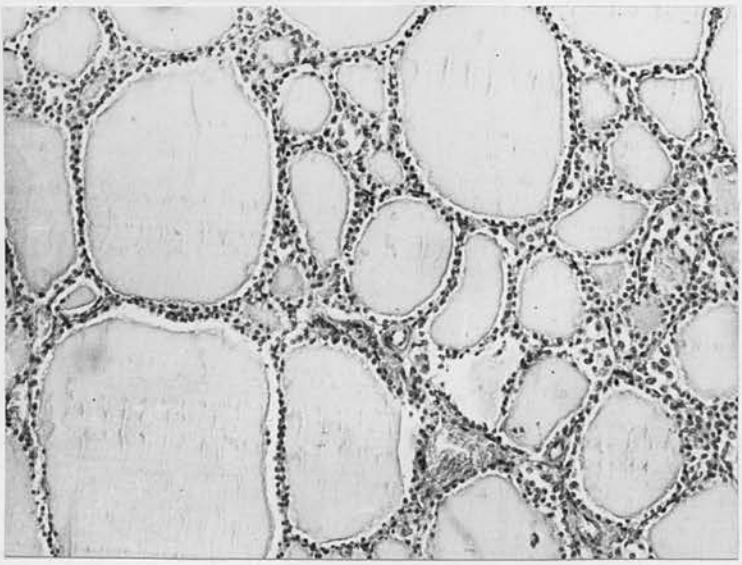

Fig. 42. Normal human thyroid gland from a woman aged 45, who died from a massive subarachnoid haemorrhage. Thyroid gland weighed 18 g. Section shows range of follicular size found normally.
H. & E. x 125.

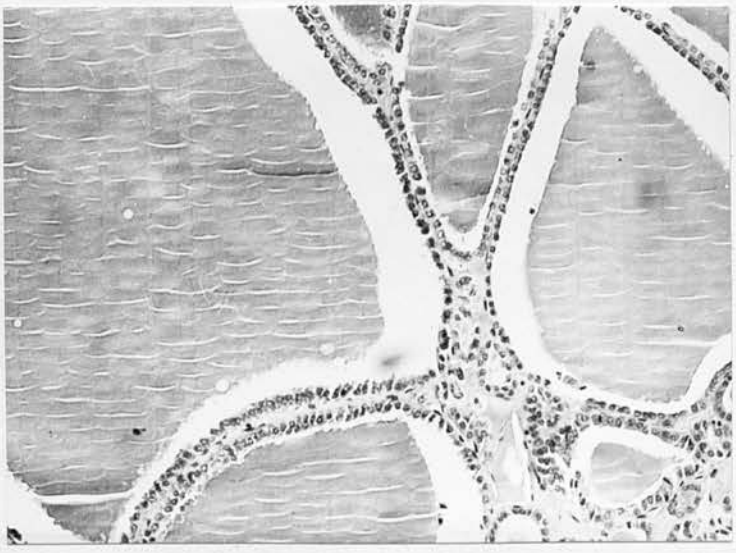

Fig. 43. Female M.S. Simple goitre, with gland about 80 g. Section shows very wide range of follicular size, with macrofollicles predominating. H. & E. x 125.

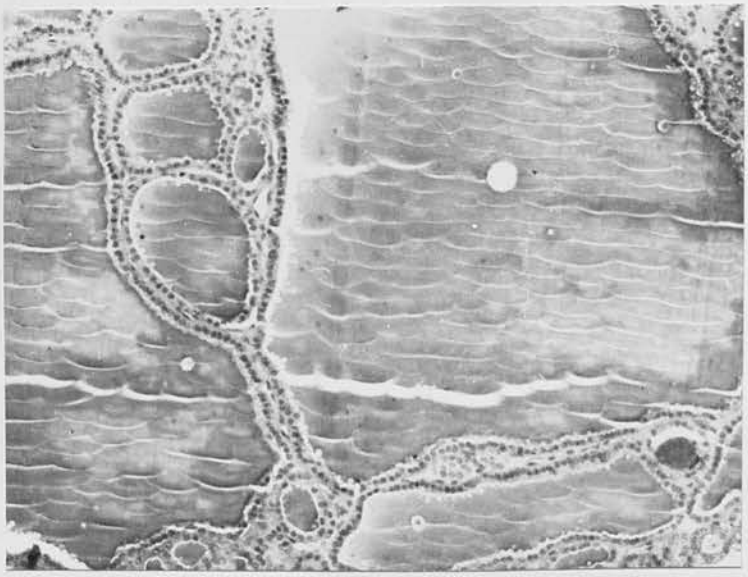

Fig. 44. Female F.B. Simple goitre, with gland about 100 g. Section shows wide range of follicular size with macrofollicles predominating. H. & E. x 125.

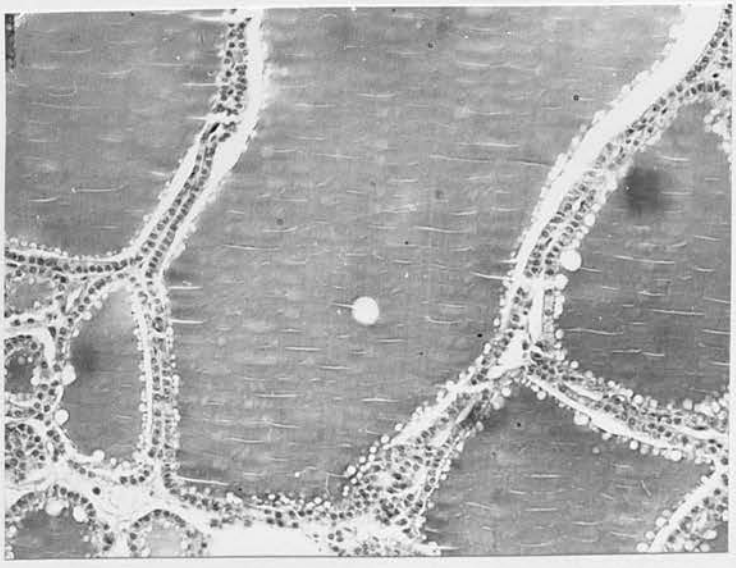

Fig. 45. Female W.F. Simple goitre, with gland about 175 g. Section shows wide range of follicular size, with macrofollicles predominating. H. & E. x 125.

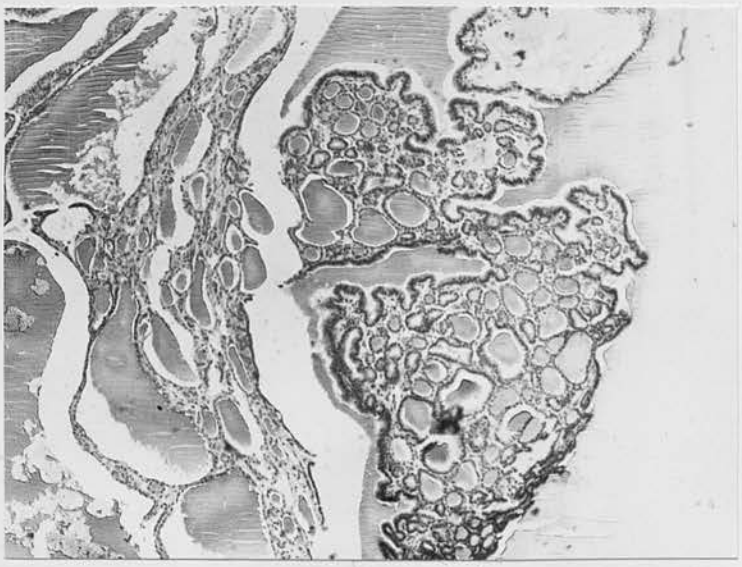

Fig. 46. Female M.S. Simple goitre, with gland about 80 g. Section shows papillary hyperplasia protruding into a macrofollicle.
H. & E. x 50.

Fig. 47. Female A.D. Simple goitre, with gland about 60 g. Section shows fibrosis around a macrofollicle, and epithelium appears to be involuted.

H. & E. x 125.

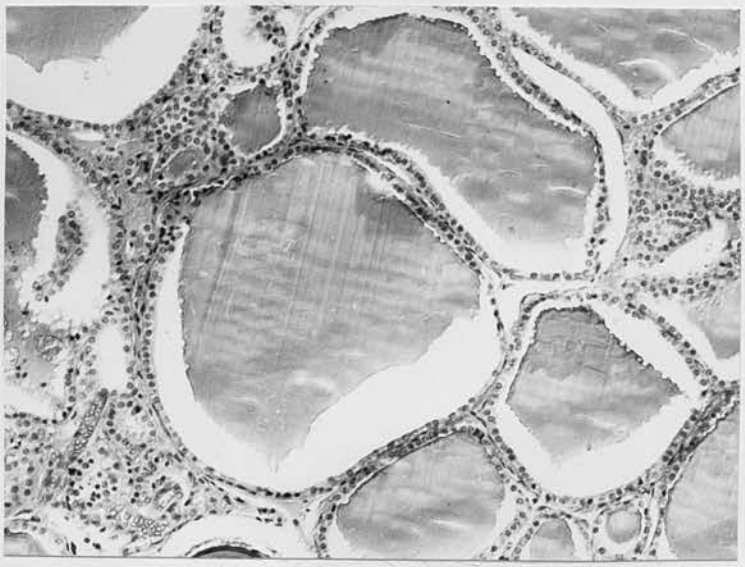

Fig. 48. Female B.M. Simple goitre, with gland about 60 g. Section shows a normal range of follicular size, but there is considerable non-acinar epithelium between follicles. H. & E. x 125.

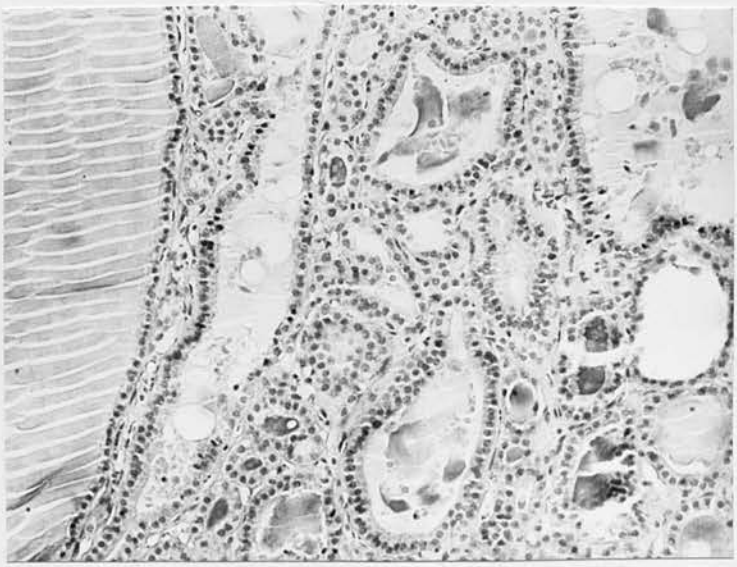

Fig. 49. Female I.S. Simple goitre, with gland about 100 g. Section shows much non-acinar and poorly formed acinar epithelium beside a macrofollicle. H. & E. x 125.

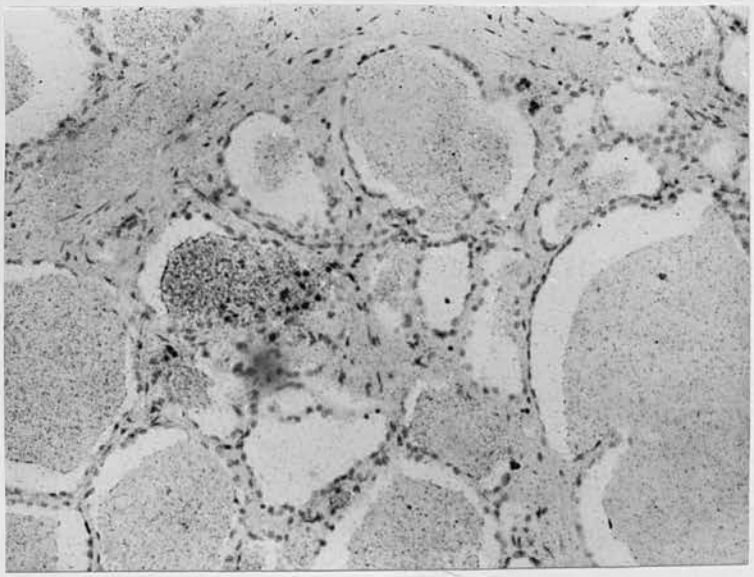

Fig. 50. Autoradiograph. Female A.D. Section shows non-uniform uptake of ^{131}I, with blackening in one small follicle, and little surrounding activity.
Haematoxylin x 125.

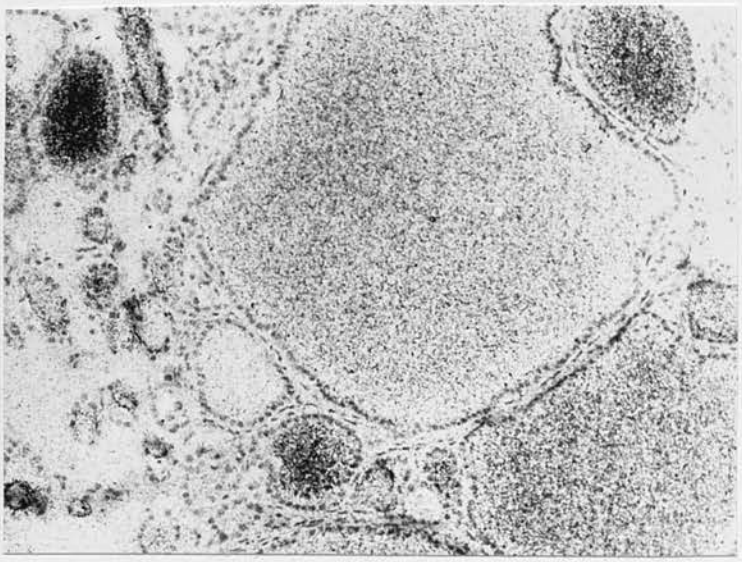

Fig. 51. Autoradiograph. Female I.S. Section shows non-uniform uptake of ^{131}I, with intense blackening of macrofollicles.
Haematoxylin x 125.

Discussion

The results of these investigations in patients with goitre in Sheffield can be most fruitfully discussed in relation to the three main aetiological factors that may operate in simple goitre. Deficient intake of iodine has long been recognised to be associated with high goitre incidence, and it is true that very few goitrous areas of the world have been recognised, without there being some evidence of deficient supply of iodine in food and water. This evidence is now extensive, convincing, and may be summarised as follows.

Deficient iodine intake has been shown directly by low levels of iodine in soil in goitrous areas (4,206,149, 362), by low iodine content of drinking water (149, 206, 158), and by low iodine content of the diet measured either by chemical analysis of the food or indirectly by dietary surveys and use of tables of iodine content of foods (357, 358, 206). Discrepancies between goitre incidence and iodine contents from many different parts of the world have been noted. In the United Kingdom, goitre was found in many parts of England when the iodine content of water was above 1 µg./litre and very rarely in Scotland even with the content below 1 µg./litre (256). It is also striking that iodine content of milk does not reflect the iodine content of pasturage, as shown by Young *et al.*(395) for Somerset and Suffolk. Costa *et al.* (80) were unable to demon-

strate an inverse correlation between iodide intake and goitre incidence in Italy. The work of Hercus and co-workers (146, 147, 148, 149) in New Zealand presents a most convincing correlation of goitre with reduced iodine in soils, and reduced intake in food. Their mean iodine intake for normal persons was 40·3 µg./day, and 26·0 µg. for goitre patients. The most striking point apart from the difference is that goitre was absent on an intake of 40 µg. Discrepancies in this work are also present. Girls with goitre were found to have a higher urinary excretion of iodine than a corresponding group without goitre (147). These workers also found a seasonal variation in iodine excretion, in that this was greater in the spring than in the autumn (149). Similarly Virtanen and Virtanen (359) in Finland found the intake of non-goitrous adults to be 65µg., and 55 µg. in goitrous patients. The corresponding results for urinary excretion were 25 µg. and 21 µg. Koutras et al. (181) recently found the iodine intake in goitrous patients to be 75 µg./day compared to 116 µg. in a normal group. Urinary iodine excretion was 59 µg. and 91 µg. Thus goitrous patients in Glasgow had higher intakes and excretions than non-goitrous persons in New Zealand and Finland. This discrepancy is found in many other published results on urinary iodine excretion in goitre. Of particular

interest is the wide range of iodine excretion in patients without goitre.

The results of Stanbury *et al.* (324) in Mendoza, and Querido *et al.* in the Netherlands (281, 339) and Lamberg *et al.*(186) in Finland show reduced urinary excretion of iodine in goitre patients, and a significant correlation exists between the uptake of ^{131}I by the thyroid and the iodine excretion, in that high uptake is found with low excretion. However, this correlation is only obvious below a urinary excretion of 40 µg. Above this there is no significant correlation. High uptake of ^{131}I by the thyroid is an almost constant finding in simple goitre, and is confirmed for goitre in Sheffield. This is usually regarded as a result of iodine deficiency producing low plasma inorganic iodine. Koutras *et al.* (181) produce evidence that mean plasma inorganic iodide is 0·07 µg./100 ml. in goitre and 0·30 µg./100 ml. in a normal group. However Riggs (292) has shown that under equilibrium conditions, the plasma inorganic iodide is equal to the urinary excretion of iodide divided by the renal clearance of iodide. There is thus a linear relationship between total excretion in urine and plasma concentration, provided that renal clearance is unchanged, and this was shown to be so by Koutras *et al.* From the data given by Koutras *et al.* (181) the plasma iodide concentration should be 0·20 µg./

100 ml., calculated on the excretion data and not 0·07 µg./100 ml. as found. These calculations suggest some technical error in these results. Also it is only an assumption that such measurements of plasma iodide concentrations reflect the average concentration during each 24 hr. It is entirely possible that increased thyroid uptake following a temporary period of block by antithyroid agents, will temporarily lower plasma iodide. It is of interest that these measurements were made when the patients were fasting.

It can be contended that demonstration of reduced iodine intake on excretion in goitre patients compared to non-goitrous patients, does not demonstrate that the reduced intake is the sole cause of the goitre, and indeed much of the evidence already given supports this. This is not to deny that severe and prolonged iodine deficiency will produce thyroid enlargement. As shown by Riggs (292)

$$M_G = \frac{1{\cdot}19\ C_k H}{\emptyset P (In - F)}$$

where M_G = Mass of active thyroid tissue

C_k = Renal plasma clearance of iodide

H = Rate of secretion of hormonal iodide by the thyroid

$\emptyset$ = Blood flow through the thyroid gland

P = Extraction efficiency of the thyroid gland

In = Daily intake of iodide

F = Rate of excretion of organic iodide

Thus if all other functions remain unchanged, the thyroid mass will increase linearally with decreasing intake of iodine. The evidence from Iceland (311), that thyroid mass decreases with increased iodine intake supports this argument. Unfortunately it is not known what thyroid size is found with various known intakes. It seems reasonable to accept 100 µg. of iodine as an intake which is not associated with increased thyroid size. This means that the size will be less than 25 g., from work already discussed. Intake could then fall to 50 µg. and thyroid size would only increase to around 40 - 50 g. Thus moderate deficiency could be present without large thyroid glands. In addition it is possible for the extraction efficiency of the thyroid to increase, as normally it is only 0·2. This function is difficult to measure and there is no published work on its measurement in thyroid diseases. There is much evidence that the thyroid gland can cope with moderate iodine deficiency without thyroid enlargement. Roche (294) in Venezuela has shown that non-goitrous patients living in regions of iodine deficiency may have as high uptakes of ^{131}I as patients with goitre. These patients also have low urinary iodide excretion. Similarly experimental iodine deficiency reported by Money et al. (249), Axelrad, et al., (23), Taylor and Poulson (338), and Van Middlesworth (353),

shows that iodine deficiency must be severe before thyroid enlargement is produced and that even then it may take up to three months for enlargement to develop. Thus the demonstration that goitre is associated with reduced iodine intake or excretion, is not convincing proof that this deficiency is the only cause of the goitre. This is probably so when the deficiency is very severe and prolonged, but in most areas studied, there have been people living in the same conditions without thyroid enlargement.

Theoretically goitre incidence should diminish with iodine supplementation, if iodine deficiency is contributing to the production of the goitre. The considerable evidence on this point has already been discussed in chapter 3 and some discrepancies noted. The conclusion of Hercus and Purves (148) in 1936, that simple goitre has not been demonstrated, unless intake is below 150 µg.I/day, is still broadly true, with only rare exceptions (70, 309, 343).. However, many regions are free from goitre with intakes much below this, as Hercus and co-workers showed (149).

Low thyroidal concentration of iodine was found without exception in the cases of simple goitre studied in Sheffield. This has been the usual finding by other workers and given as evidence supporting iodine deficiency as the cause of goitre. Hercus et al.

(147) found the concentration in goitre to be between 0·06 - 0·42 mg./g. (dry wt.) whereas the mean value in Sheffield was 0·47 mg./g. Their normal value was 2·1 mg./g. Similar findings were reported by Marine and Lenhart (236) and Stanbury et al. (324). It is striking that the total iodine content of these glands is usually normal, and many glands have very high total iodine contents. Thus in Mendoza, Stanbury et al. found 17 mg. of iodine in one gland. In Sheffield the highest value was 20 mg. It seems surprising that a compensatory mechanism should be so inefficient that the mass of thyroid has to be greatly increased, with a normal or higher than normal total iodine content. This lends support to the hypothesis that there is some block to the binding of iodine by the glands, producing a lowered concentration of iodine, as would occur with goitrogenic substances like thiouracil. Stanbury et al. (324) in Mendoza also remark on the high total thyroid iodine and suggest that this is the most puzzling and challenging observation of their study.

Inborn and often genetically determined defects of thyroid hormone synthesis have recently been recognised as the mechanism of some cases of congenital goitre (325,214). McGirr (213) has suggested that minor degrees of such abnormalities may explain many cases of simple goitre developing in

later life. It is probable that iodine deficiency would exaggerate such defects. These defects are three in type. The first shows a defect in the binding of iodine to tyrosine radicals, and this is demonstrable by discharging radioiodine from the thyroid by perchlorate. Such a defect was not present in simple goitre in Sheffield and Morgans and Trotter (251) have noted its lack in simple goitre and also Cottino et al. (82). The second type is postulated to be due to lack of a dehalogenase in the thyroid and possibly generally in the tissues. Carr et al.(58) have found normal dehalogenase activity in simple goitre. The third type shows increased concentrations of radiomonoiodotyrosine in the thyroid and reduced concentrations of radiothyronines especially thyroxine. The work of Pit-Rivers et al.(274) would seem to support the claim that a hypothetical enzyme defect may prevent coupling of tyrosines to thyroxines in simple goitre. They showed high ratios of monoiodotyrosine to diiodotyrosine, and reduced thyroxines in cases of simple goitre. However these findings are not specific. They have been found in the presence of iodine deficiency by Querido et al. (281). Bois and Larsson (41), and Bastenie et al. (28). It will be demonstrated in the present work that such findings also occur from the activity of goitrogenic

substances similar in type to thiouracil. That there is considerable evidence that the occurrence of such substances in food may play a part in the production of simple goitre will be discussed with the experimental results described in the following chapters of this work.

Summary

1., The mean 48 hr. thyroid uptake of ^{131}I in 194 patients with simple goitre was 50·1 ± 0·86%, and 43·1 ± 0·76 in 180 subjects without goitre.

2. A significant fall in uptake with increasing age was found in both groups, and the slope of the parallel regression lines for these groups was -0·28.

3. Mean serum protein bound iodine in 96 patients with simple goitre was 5·18 ± 0·11 µg./100 ml., and 5·81 ± 0·09 µg./100 ml. in 95 patients without goitre. This difference was significant (P 0·01), and the respective ranges were 2·8 - 7·4 µg., and 4·2 - 7·3 µg.

4. In 20 patients with simple goitre, the mean total iodine content of the thyroid was 7·7 ± 1·0 mg. Total thyroidal iodine increased and concentration of thyroidal iodine decreased significantly with increasing thyroid size.

5. Mean renal plasma clearance in 22 patients with simple goitre was 29·7 ± 2·5 ml./min., and mean biological half-life of thyroidal ^{131}I was 258 ± 42·7 dy.

6. No defect in organic binding of ^{131}I was demonstrated in simple goitre.

PART II

EXPERIMENTAL STUDIES OF FACTORS INFLUENCING THYROID SIZE

5

EFFECT OF SEASON ON THYROID SIZE

In many areas of the world with high incidence of simple goitre, there are reports of thyroid enlargement in animals. In New Zealand Hercus et al., (146, 147) have noted goitre in sheep, cattle and pigs, and episodic goitre in new-born lambs has also been reported (313, 314, 393). Haarasen (136) has noted variation in pig thyroid weights in Finland, and Peltola and Vartiainen (266) were unable to prevent thyroid enlargement in cattle with iodine supplementation. Nearly fifty years ago, Martin (239, 240) found large thyroid glands from sheep in England.

In an attempt to elucidate the cause of simple goitre in Sheffield, a study of sheep thyroid glands was begun in 1958, and some factors which might play a role in seasonal variation of thyroid weight were studied.

Seasonal variation in sheep thyroid glands

Regular collections of sheep thyroid glands were obtained from the Sheffield abattoir. The larynx and pharynx with several inches of attached trachea and oesophagus were removed from the sheep, immediately after death, by the local slaughtermen after collection. The thyroid glands were dissected from the tissues. They were weighed fresh, then dried and

iodine content measured. Body weights of the sheep, after removal of viscera and skin were obtained from the abattoir. Thyroid glands from approximately 30 sheep were collected nearly every week for 2 yr, and came from all parts of England north of Leicester and Nottingham. The majority of the batches of sheep had grazed in Yorkshire, with either hill, wold or pasture grazing.

The results are shown in Fig.52 , where mean thyroid wet weights are shown against date of collection. A striking seasonal threefold variation in weight is noted, with peak weight of 7 - 8 g. occurring during April and May of each year. This falls quickly during June and a basal level of 2 - 3 g. maintained between August and December. This basal level agrees with the values found by Martin (239) in England and Guyer (135) in Scotland. The values for dry weight show a similar seasonal variation. There is no correlation with body weight or grazing site. The iodine concentrations of these thyroids are shown in Fig. 53 , expressed as mg./g. dry weight and plotted by season. Obviously there is no regular variation in iodine concentration corresponding to the thyroid weight variation, and means that total thyroid iodine content increases during April and May. This is at variance with the results of Seidell and Fenger (306), who found a threefold higher iodine concentration in sheep, cattle and pig

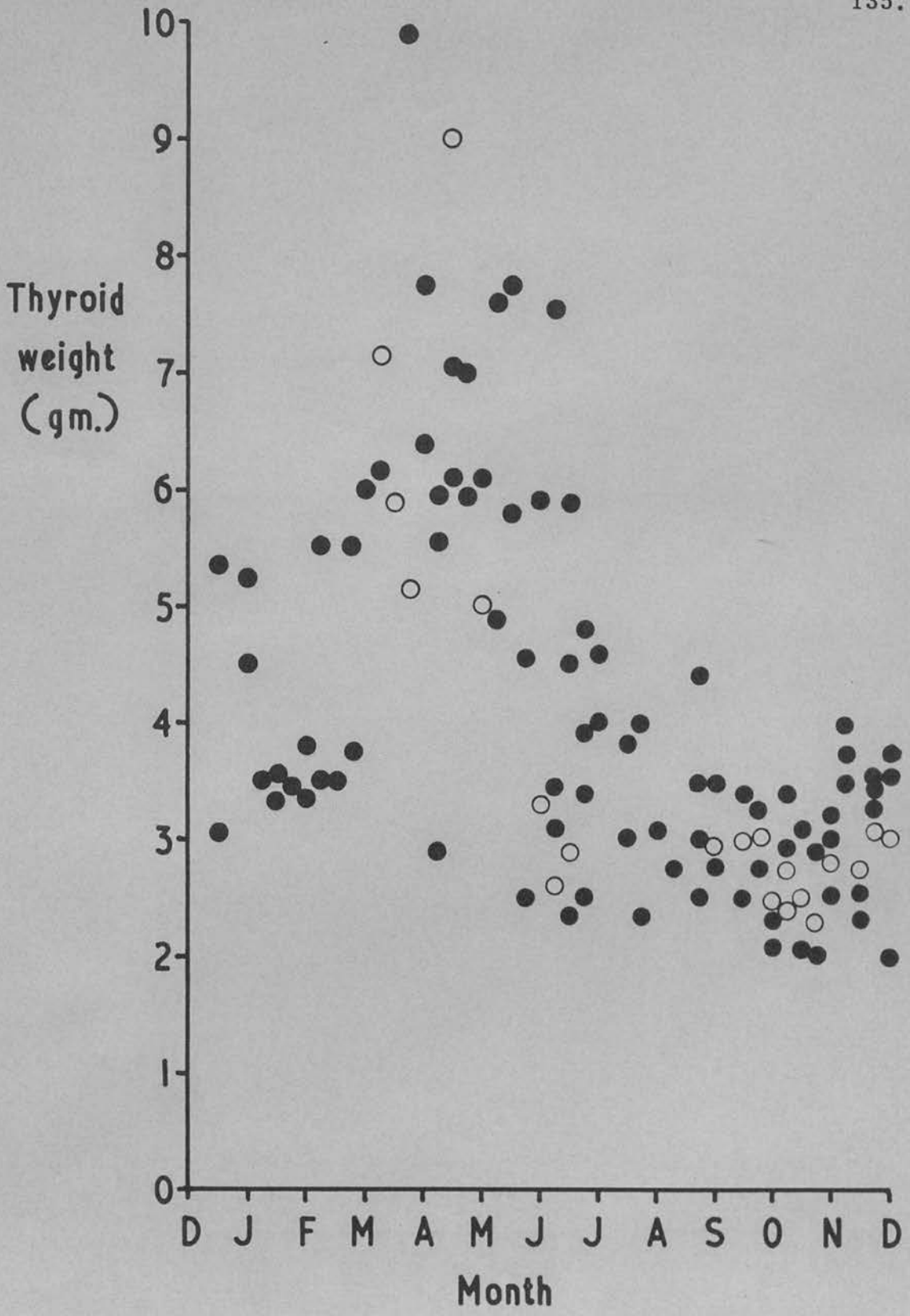

Fig. 52. Mean wet weights of thyroid glands of sheep from individual farms in England during 1958 (open circles) and 1959 (closed circles).

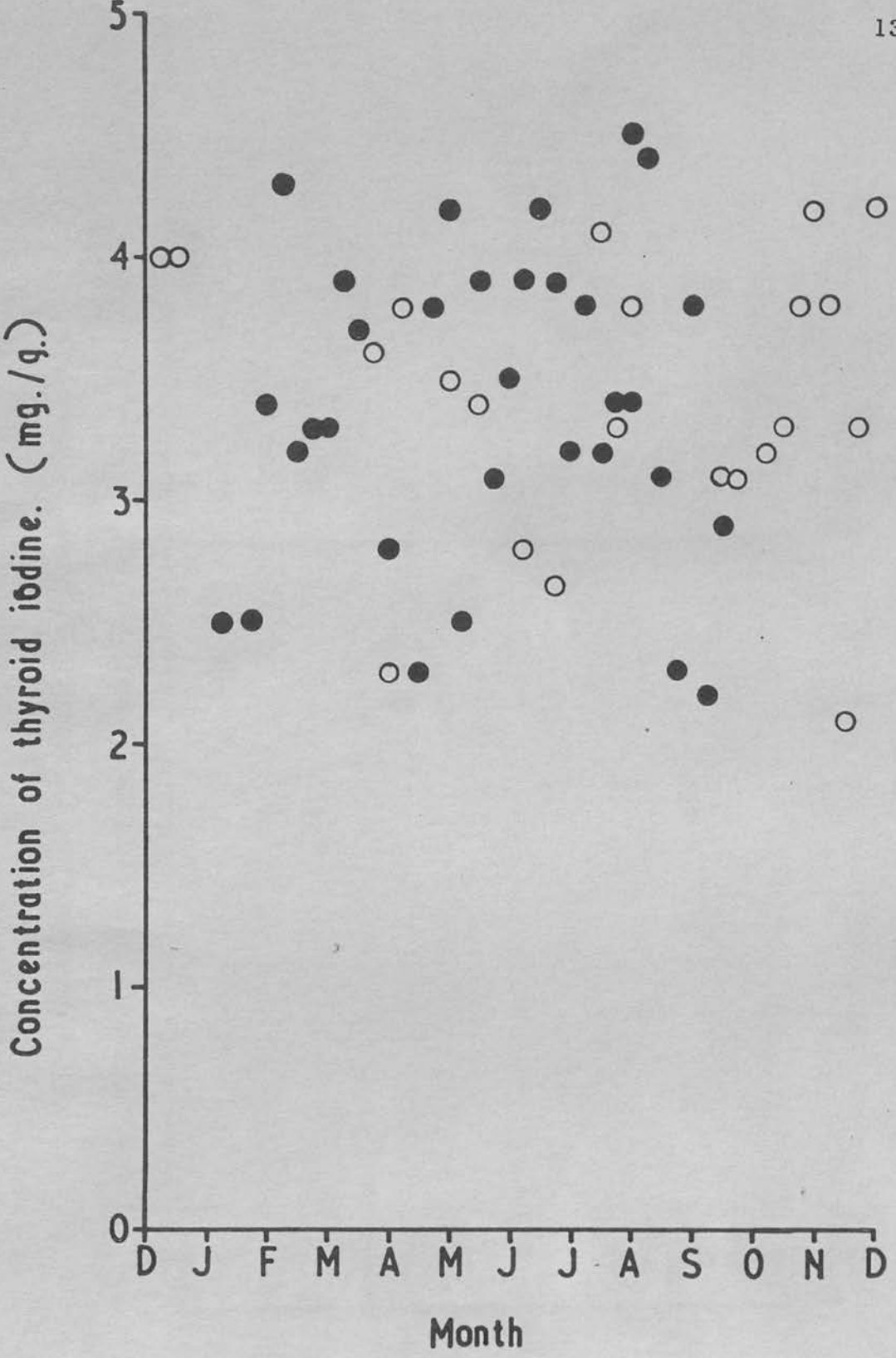

Fig. 53. Mean iodine concentrations of sheep thyroid glands (mg./g.dry wt.) from individual farms in England during 1958 (open circles) and 1959 (closed circles).

thyroids during June to November, compared to December to May. They found a similar variation in sheep and cattle thyroid weights to that presently reported.

Microphotographs of representative sheep thyroid glands during April - May at the peak of thyroid weight, and during October, when the basal level is reached, are shown in Figs. 54-57. Both are well filled with colloid, but the larger glands are more cellular, with protrusion of clumps of cells into the colloid. These may be compared with Fig. 58 , which shows a microsection of the thyroid gland of a still-born lamb, which had a goitre of 100 g. There is no colloid and it presents the picture of severe hyper-plasia. This lamb came from a flock which had been fed on rape seed for some weeks, and this is known to contain large amounts of goitrogenic material.(18).

Effect of light on thyroid activity

Duration of daily light exposure is one of the many factors which might explain a seasonal variation in thyroid activity. This was studied in mice by illuminating a control group for 16 hr. each day and another group for only 8 hr/day. This was controlled by an automatic electronic control unit, and the illumination provided by a filtered electric lamp, giving a spectrum similar to daylight. Temperature was maintained within $\pm 2^{\circ}$. In the first experiment the mice were fed on a commercial cube diet with a moderate iodine content of 250 µg. I/kg, and the

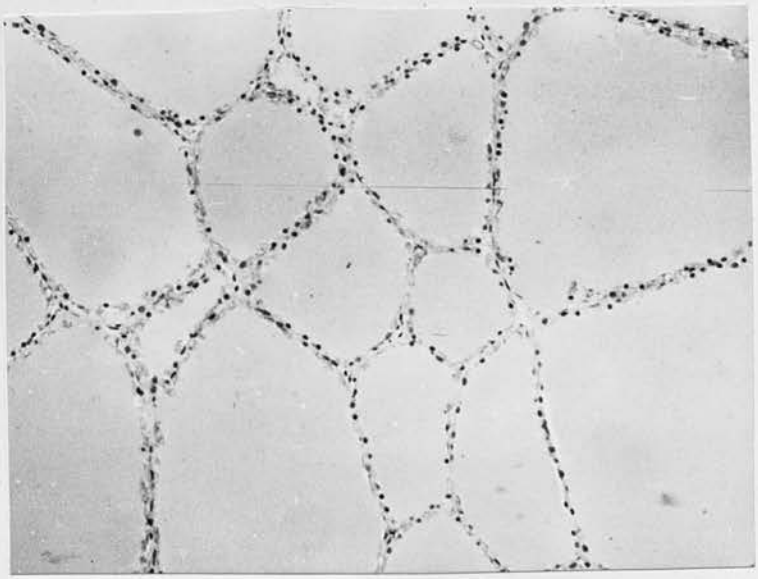

Fig. 54. Sheep thyroid gland collected in October, 1958, weight 3·2 g. Section shows resting appearance of epithelium and is to be compared with Fig. 56. H. & E. x 125.

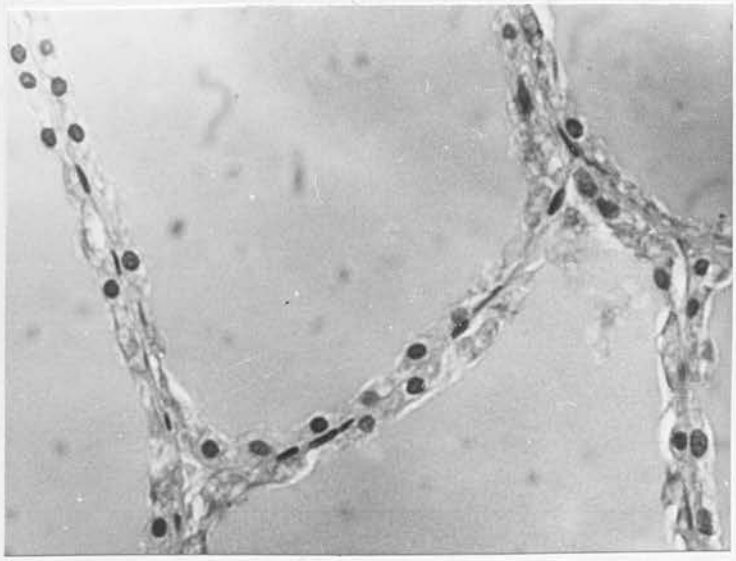

Fig. 55. Sheep thyroid gland as in Fig. 54. High power section shows resting appearance of epithelium and is to be compared with Fig. 57. H. & E. x 500.

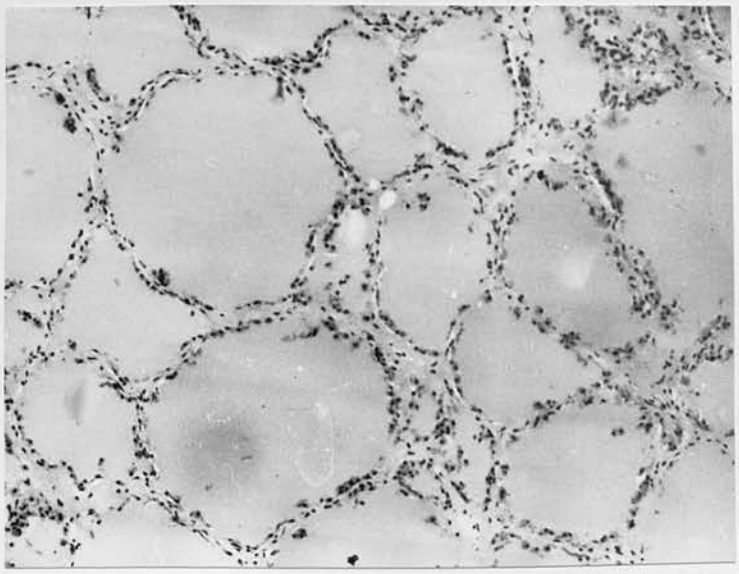

Fig. 56. Sheep thyroid gland collected in May 1958, weight 9·7 g. Section shows increased number of cells around follicles, and protrusions of epithelium into the colloid. H. & E. x 125.

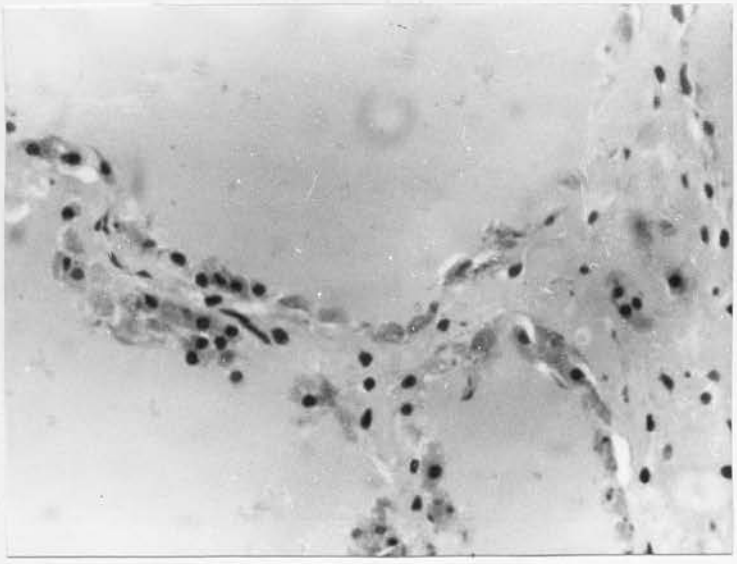

Fig. 57. Sheep thyroid gland as in Fig. 56. High power section shows the increased cellularity and cellular protrusions into the colloid. H. & E. x 500.

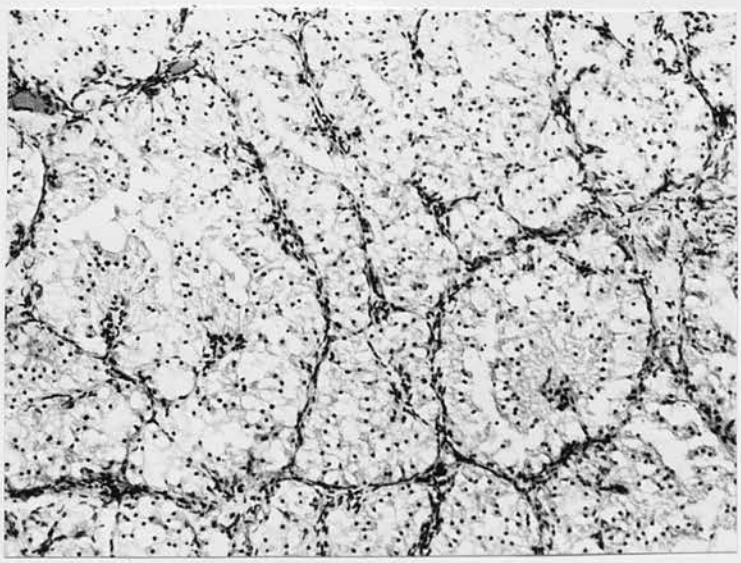

Fig. 58. Stillborn lamb, thyroid gland collected in March 1959, weight 103 g. The ewe had fed on rape for some weeks before birth. Section shows intense cellular proliferation and complete loss of colloid, though follicular structure can still be made out. H. & E. x 125.

Table 18. Effect of duration of light exposure on thyroid weight and ^{131}I uptake in mice.

Treatment	Duration (days)	Mean increase in thyroid weight as % of control group	Mean 24 hr. ^{131}I content of thyroid as % of control group	No. of mice treated	No. of controls
Darkness + moderate Iodine diet	120	1 N.S.	89 N.S.	20	16
Darkness + low Iodine diet	120	20 *	166 *	24	24

Control groups received illumination 16 hrs./day, and treated groups 8 hrs./day.

The significance of differences between control and treated animals was calculated using Student's t test and indicated thus

** $P < 0.01$; * $0.05 > P > 0.01$; N.S. $P > 0.05$

results are shown in Table 18 . The experiment was repeated using a low iodine diet of 50µg./kg. (Vims dog biscuits), and the results shown in the same table. Thyroid weight and 24 hr. uptake of ^{131}I were both significantly increased in the mice illuminated for only 8 hr./day, and given the low iodine diet. No effect was found with the high iodine diet.

Discussion

The present investigation clearly demonstrates a seasonal variation in sheep thyroid weight. The lack of correlation with body weight makes it unlikely that this is due to varying age of sheep being killed at different times of the year, at least up to mature body weight. That it might be due to different breeds of sheep is also unlikely, as there is no tendency for different breeds to be killed according to season. Assuming a real increase in thyroid weight by season, two mechanisms can be postulated. The first is that changing environmental conditions may stimulate thyroid hormone secretion rate. Henneman *et al.* (144) and Griffin (132) give evidence using the thyroid substitution method that sheep thyroid secretion rates are highest in March, and Flamboe and Reineke have shown this in goats.(105). Environmental factors known to stimulate such secretion are lowered ambient temperature, and light. Dempsey and Astwood (87) have demonstrated the thyroid stimulation produced by

lowered environmental temperature, and this has been confirmed and shown to be mediated mainly but not entirely through increased thyrotrophic secretion by many other workers (42, 47, 87, 329, 385). The effect of light is more complex, and has been reviewed by Tixier-Vidal (342). In most species studied it has an effect, and is usually stimulatory. However in others thyroid function is increased by darkness, and this applies particularly to rats and mice. This is shown to be dependent on iodine intake in the present work, and appears to be the first demonstration that relative iodine deficiency is necessary for such stimulation. It is unlikely that either of these stimuli account for the variation in sheep thyroid weight. The peak weight would occur around January if due to temperature effect, and either then or July if the stimulus were light or darkness.

A second mechanism for variation in thyroid weight may be a varying ingestion of iodine or positive goitrogenic factors. There is considerable evidence that iodine contents of plants and pasturage vary with season and that such contents are much lower with the spring growth of plants (Hercus et al. 149, McClendon, 206). As the iodine content of the thyroid increases with thyroid weight, it is unlikely that this plays a part.

Goitrogenic factors in certain plants found in

pastures are now recognised (51, 7, 108) and could produce increase in thyroid weight. Their presence in milk of grazing cattle will be reported in the following chapter.

Summary

1. A threefold variation in sheep thyroid weight has been found, with peak weight of 7 - 8 g. in April - May, and basal weight of 2 - 3 g. from July to December. There is no corresponding variation in iodine concentration of these glands.

2. Reduced duration of exposure to light produces thyroid changes in mice, manifest by increased thyroid weight and uptake of ^{131}I. This only occurs when iodine intake is low.

6

GOITROGENIC ACTIVITY OF MILK

Considerable evidence on the occurrence of natural goitrogens has accumulated since the accidental demonstration by Chesney *et al.* (65, 371, 372) that cabbage produced simple goitre in rabbits. Goitrogenic activity has been found in seeds of a number of *Brassica*, by Hercus and Purves (148) and Kennedy (171), and Greer (129) confirmed that this activity varied from batch to batch, and seasonally in the same batch. Astwood *et al.* (18) isolated this activity as 1-5-vinyl-2-thioonozolidone, from seeds of a number of *Brassica*, and the roots of turnips and swedes. Virtanen *et al.* (360) and Altamura *et al.* (6) later showed that this compound was present in the leaves of fresh cabbage in concentrations up to 100 µg./kg. Greer and Astwood (130), and Fertman and Curtis (102) assayed the antithyroid activities of foods and found that milk was the most active of animal products tested. Flux *et al.* (108) and Butler *et al.* (51) have demonstrated goitrogenic activity in white clover (*Trifolium repens L.*) and shown that this may be partly or entirely due to its cyanide content, with conversion to thiocyanate in an animal feeding on it. The main source of such goitrogens in plants and vegetables, apart from direct ingestion by man, will be that which is present in cow's milk. A study of such milk was undertaken in Sheffield, using

fresh milk initially and later dried milk.

Effect of milk on uptake of radioiodine

(a) Effect on man. During March and April, 1959, the effect of 500 ml. of fresh milk on the accumulation gradient of ^{131}I, as defined by Stanley and Astwood (327), was measured in six female patients who had normal thyroid function and no thyroid enlargement. Radioiodine measurements had been requested to eliminate the possibility of hyperthyroidism, and their symptoms were due to anxiety. Ten μc. of ^{131}I was given intravenously and thyroid content of ^{131}I measured every 20 min. After 2 1/4 hr. 500 ml. of fresh milk, obtained from a single supplier in Sheffield, was drunk within 15 - 20 min., and measurements continued for a further 3 hrs. The results are shown in Fig. 59 . There was no effect in one subject, questionable inhibition corresponding to Grade 1 of Stanley and Astwood (327) in 3 subjects, and definite but incomplete inhibition (Grade 2) in 2 subjects. It is possible that this variable effect is due to varying sensitivity of the subjects to any antithyroid substances in milk, or to varying amounts in the milk. These results are similar to those given by Clements and Wishart (71). Greene, Farran and Glascock (122) obtained little effect from 1 pint of milk tested in this way, but only measured gradient for 20 min. after ingestion

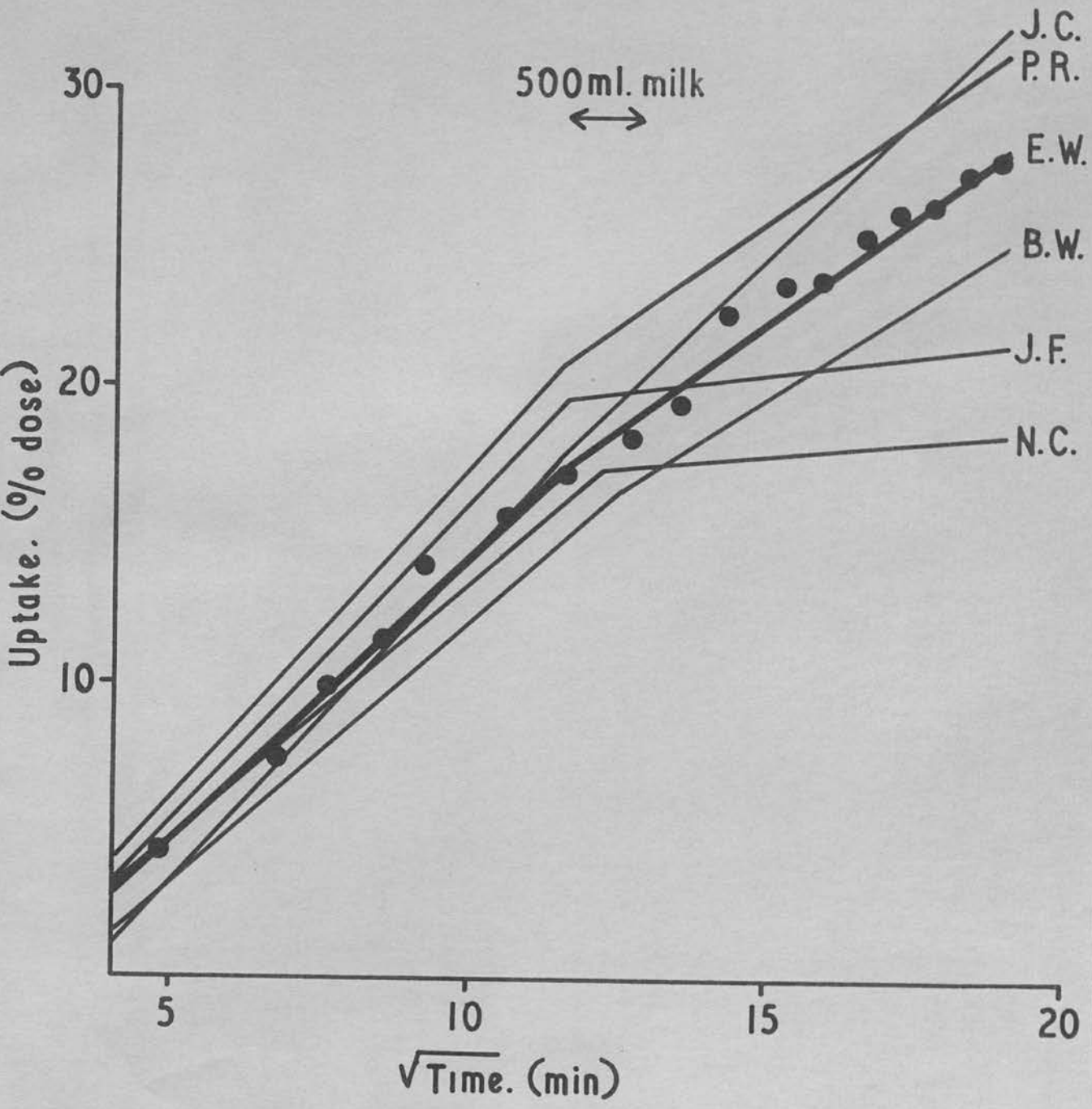

Fig.59. Effect of 500 ml. of fresh milk during March and April 1959 on the accumulation gradient of ^{131}I given intravenously. Milk was drunk by six female patients, who had no thyroid enlargement, between 2 hr. and 2½ hr. after ^{131}I. There was no inhibition of accumulation rate in J.C., Grade I inhibition in P.R., E.W., and B.W., and Grade II inhibition in J.F. and N.C. For clarity the individual points are shown only for E.W.

of the milk. It is very unlikely that any contained antithyroid substances would be absorbed within this period.

(b) Effect on rats. Fasting rats weighing 100-120 g. were given 5 ml. of fresh milk by stomach tube and this dose was repeated after 1 hr. Control rats were given water and 1 hr. after the second dose all were given 0·5 μc. of ^{131}I intraperitoneally. Rats were killed 4 hrs. after ^{131}I and thyroids removed. Fresh milk was obtained from the same supplier as above. Later dried milk, manufactured by Glaxo Ltd. from milk obtained from East and North Ridings of Yorkshire was also tested. This dried milk was made up in warm distilled water and given as two doses of 5 ml., each of which contained 1·25 g. of dried milk powder. This concentration is approximately double the total solids in fresh liquid milk.

Between March and May, 1959, liquid milk produced almost 50% depression of 4 hr. uptake of ^{131}I in rats, as shown in Table 19 and/ Fig. 60. No significant effect was found on monthly testing until March 1960, when antithyroid activity in milk continued until May, 1960. The effect was significantly less in 1960 than in 1959. Dried milk produced depression of uptake almost throughout the year November 1959 to November 1960, as shown in Table 20 . However it should be pointed out that the dried milk test procedure was equivalent to giving twice the dose

Table 19. Antithyroid activity in rats of fresh liquid milk from Sheffield as tested by two oral doses of 5 ml. separated by 1 hr.

Date of test	Mean Depression of ^{131}I content of thyroid as % of control group	No. of rats treated	No. of control rats
16.3.59	40**	8	8
24.3.59	45**	8	8
21.5.59	21*	10	10
1. 6.59	17 N.S.	8	6
6. 7.59	3 N.S.	8	8
9. 9.59	6 N.S.	8	8
21.10.59	2 N.S.	8	8
4.12.59	13 N.S.	8	8
6. 1.60	16 N.S.	8	8
17.2.60	3 N.S.	10	10
4. 3.60	-2 N.S.	8	8
16.3.60	17*	8	8
8. 4.60	23*	12	12
13.4.60	22*	10	10
25.4.60	28**	10	10
12.5.60	18*	8	8
28.5.60	11 N.S.	8	8

The significance of differences between control and treated animals was calculated using Student's t test and indicated thus

** $P < 0.01$; * $0.05 > P > 0.01$; N.S. $P > 0.05$

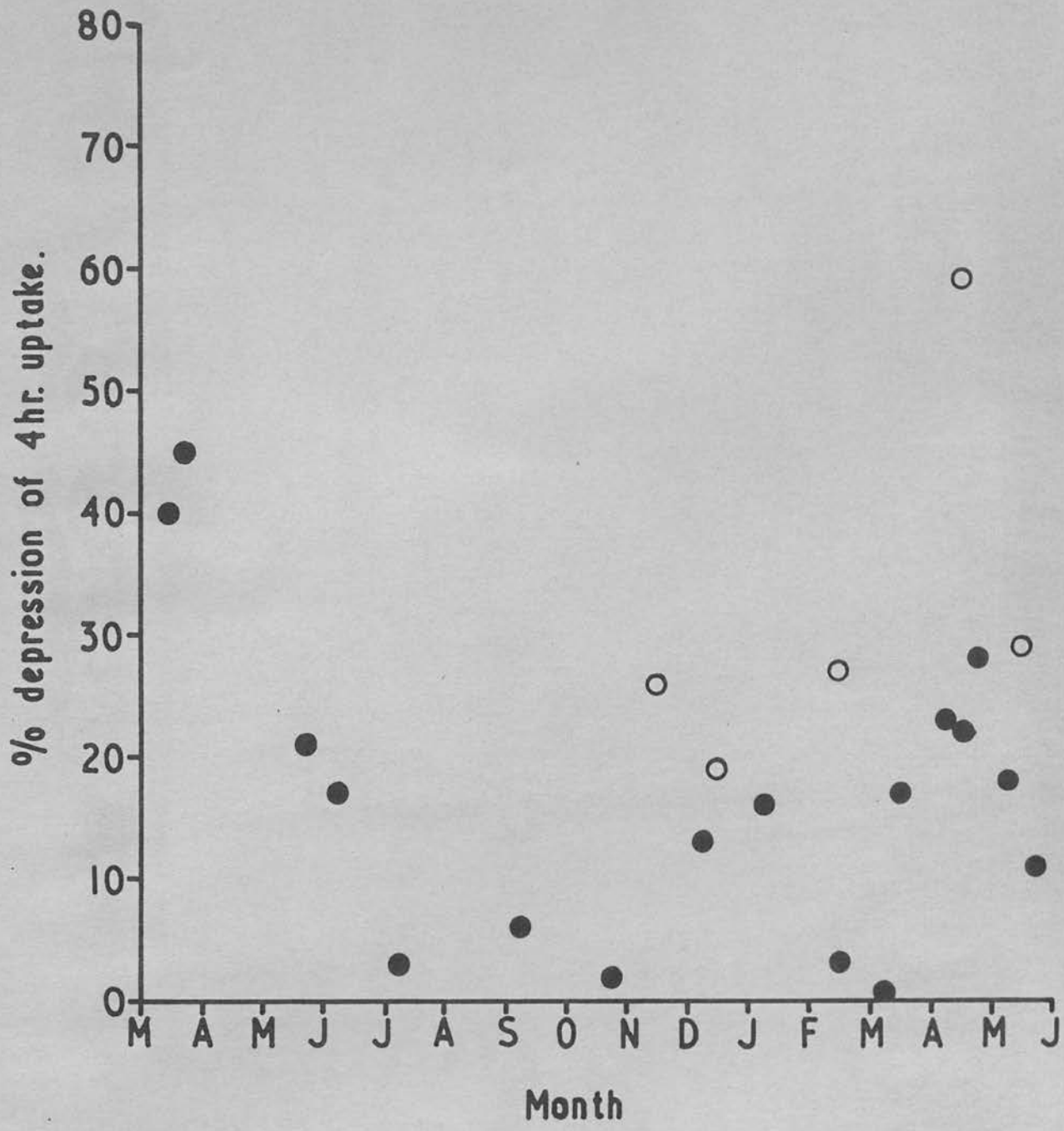

Fig.60. Depression of 4 hr. uptake of ^{131}I in rats by fresh milk (closed circles) and dried milk (open circles), during 1959 and 1960. Liquid milk was given as two oral doses of 5 ml. and dried milk by two oral doses of 1·25 g., both separated by an interval of 1 hr. One hour after second dose ^{131}I was given intraperitoneally.

Table 20. Antithyroid activity in rats of dried milk from Yorkshire as tested by two oral doses of 1·25 g. separated by 1 hr.

Date of preparation of dried milk	Mean depression of ^{131}I content of thyroid as % of control group	No. of rats treated	No. of control rats
November, 1959	26*	8	8
December, 1959	19*	8	8
February, 1960	27*	8	8
April, 1960	59**	10	10
May, 1960	29**	10	10
June, 1960	33**	8	8
July, 1960	32**	8	8
August, 1960	24*	8	8
September, 1960	18 N.S.	10	10
October, 1960	15 N.S.	8	8
November, 1960	19*	8	8

** $P < 0.01$; * $0.05 > P > 0.01$; N.S. $P > 0.05$.

used in the liquid milk experiment. With dried milk there was more activity during April to July, and the batch produced in April 1960 gave almost 60% depression of ^{131}I uptake.

Goitrogenic effect of milk

A goitrogenic effect of any substance can only be conclusively demonstrated by producing an increase in thyroid gland weight. Accordingly long term experiments with milk were carried out using rats. They were given fresh milk to drink and this milk was renewed daily and obtained from the same supplier. The amount drunk was measured, as was the water that the control rats were given. Both were given the same commercial cube diet, whose iodine content was 250 µg./kg. and supplied about 2·5 µg. of iodine daily to each rat. Iodine supplementation was given in some experiments. At the end of each experiment the rats were given ^{131}I 24 hr. before being killed and while still drinking milk. Dried milk was also tested in thisway, and was made up to double the concentration of fresh milk as with experiments of depression of 4 hr. uptake.

The results of these experiments are shown in detail in Tables 21-25 and summarised in Table 26. Fresh milk given daily during the period February to March 1959, and March to May, 1959 produced significant increases in thyroid weight, expressed as mg./100 g. body weight, compared to control

animals. Iodide supplementation was given to rats drinking milk between March - May 1959 and increase in thyroid weight still occurred. Unfortunately milk without added iodide was not tested during this period. Though the added iodide did not prevent goitre from the milk, it is possible that goitre from milk alone during this period might have been greater. A very striking increase in uptake was produced by milk and iodide during this period. The goitrogenic activity of fresh milk during the period May - June 1959 was much less and was completely prevented by supplements. It is difficult to explain the depression of uptake in the iodide treated group, as there was no significant change in the group without iodide. This point will be discussed later. This reduced activity during May-June agreed with the acute experiments on depression of uptake (Table 19).

Goitrogenic activity of the dried milk prepared in April 1960 was very striking in two experiments, and agreed with acute results on depression of 4 hr. uptake. Two points should be made about these experiments. Iodide supplementation significantly reduced the thyroid hyperplasia produced by the dried milk alone, but the goitrogenic effect was not abolished. Secondly there was no significant effect on 24 hr. ^{131}I uptake at the end of the experiment with

Table 21. Effect of fresh milk with and without added iodine, on thyroid weight and ^{131}I uptake in rats

Rats fed moderate iodine diet but drinking	Duration of experiment (days)	Mean thyroid weight		24 ^{131}I content of thyroid on last day (% dose ± s.e.)	No. of Rats
		mg. ± s.e.	mg./100 g. body wt. ± s.e.		
Fresh milk during Feb.-March 1959.	30	18·0 ± 0·76	8·6 ± 0·31	10·5 ± 0·36	12
Water during Feb.- March 1959.	30	13·2 ± 0·76	5·7 ± 0·31	11·2 ± 1·21	12
Fresh milk March-May 1959. + added * iodine	42	16·5 ± 0·82	7·0 ± 0·44	3·9 ± 0·36	8
Water March-May 1959 + added * iodine	42	7·6 ± 0·52	4·7 ± 0·31	2·1 ± 0·26	8

* Iodine supplement was 200 µg. potassium iodide sub-cutaneously every 3rd day.

Table 22. Effect of fresh milk with and without iodine supplementation on thyroid weight and ^{131}I uptake in rats fed a moderate iodine diet, during the period May - June, 1959.

Rats fed moderate iodine diet but drinking	Mean thyroid weight after 42 days treatment (mg. ± s.e.)	Mean thyroid weight after 42 days treatment (mg./100 g. ± s.e.)	24 hr. ^{131}I content of thyroid on last day (% dose ± s.e.)	No. of Rats
Fresh milk (25 ml./100g. /day)	20·8 ± 0·89	8·4 ± 0·32	17·8 ± 1·43	20
Fresh milk + iodine * supplement	18·2 ± 0·76	7·9 ± 0·31	10·4 ± 1·19	20
Water (20ml./100g. /day.	18·4 ± 0·81	7·3 ± 0·32	17·3 ± 1·36	20

* Iodine supplement was 200 µg. potassium iodide subcutaneously every 3rd day.

Table23. Effect of dried milk prepared in April, 1960 on thyroid weight and ^{131}I uptake in rats, when given daily for 54 days.

Rats on moderate iodine diet and taking	Mean thyroid weight after 54 days treatment (mg. ± s.e.)	Mean thyroid weight after 54 days treatment (mg./100 g. ± s.e.)	24 hr. ^{131}I. content of thyroid on last day (% dose ± s.e.)	No. of rats
Dried milk (3 g./100g. / day)	30·8 ± 1·41	13·6 ± 0·68	10·4 ± 0·89	12
Dried milk + iodine* supplement	25·4 ± 1·19	11·2 ± 0·51	7·9 ± 0·73	6
Water (20ml./100g. /day)	18·8 ± 0·93	6·4 ± 0·32	11·2 ± 0·82	12
Water + Iodine*	17·4 ± 0·87	6·5 ± 0·31	6·5 ± 0·58	6

Dried milk was reconstituted as 125 g./l. of water and given instead of water.

* Iodine supplement was 10µg./100g./day, given as potassium iodide in the reconstituted dried milk or in drinking water.

Table 24. Effect of dried milk prepared in April, 1960, on thyroid weight and ^{131}I uptake in rats, when given daily for 110 days

Rats on moderate iodine diet and taking	Mean thyroid weight after 110 days treatment		24 hr. ^{131}I content of thyroid on last day (% dose ± s.e.)	No. of rats
	(mg. ± s.e.)	(mg./100g. ± s.e.)		
Dried Milk (3g./100g./day)	30·6 ± 1·23	9·2 ± 0·41	9·5 ± 1·10	6
Dried Milk + Iodine	21·4 ± 0·88	6·3 ± 0·29	5·4 ± 0·53	6
Water (20ml./100g./day)	20·7 ± 0·56	6·3 ± 0·19	10·2 ± 0·98	6
Water + Iodine	18·2 ± 0·72	5·2 ± 0·21	5·2 ± 0·61	6

Table 25. Effect of dried milk prepared in September 1960, on thyroid weight and ^{131}I uptake in rats, when given daily for 50 days

Rats on moderate iodine diet and taking	Mean thyroid weight after 50 days treatment		24 hr. ^{131}I content of thyroid on last day (% dose ± s.e.)	No. of rats
	(mg. ± s.e.)	(mg./100g. ± s.e.)		
Dried Milk (3g./100g./day)	17·9 ± 0·87	10·5 ± 0·51	8·0 ± 0·93	12
Dried Milk + Iodine	15·3 ± 0·82	8·5 ± 0·46	5·4 ± 0·62	12
Water (20ml./100g./day)	19·4 ± 0·91	10·8 ± 0·48	7·3 ± 0·79	12
Water + Iodine	17·1 ± 0·64	10·1 ± 0·39	5·3 ± 0·69	12

Table 26. Summary of effect of fresh and dried milk, with and without added iodine, on thyroid weight and ^{131}I uptake in rats.

Type and date of collection of milk	Mean increase in thyroid weight (mg./100g.) as % of control group	Mean 24 hr. ^{131}I content of thyroid of treated group as % of control group	Duration of experiment (days)
Fresh milk Feb.-Mar. 1959	151 **	94 N.S.	30
Fresh Milk March - May, 1959 + added iodine	148 **	185 **	42
Fresh Milk May - June, 1959	114 *	103 N.S.	42
" + added iodine	108 N.S.	60 **	42
Dried Milk April, 1960	212 **	93 N.S.	54
" + added iodine	172 **	121 N.S.	54
Dried Milk April, 1960	146 **	94 N.S.	110
" + added iodine	121 *	104 N.S.	110
Dried Milk September, 1960	97 N.S.	110 N.S.	50
" + added iodine	84 *	102 N.S.	50

** $P < 0.1$; * $0.05 > P > 0.01$; N.S. $P > 0.05$

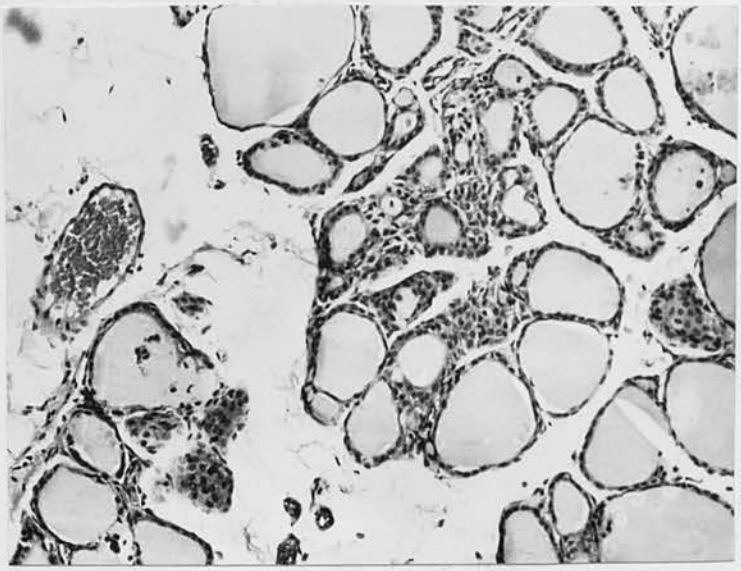

Fig. 61. Thyroid gland from a rat on a moderate iodine diet and drinking water for 110 days. Section shows the varied follicular size and height of epithelium of the normal rat thyroid.
H. & E. x 125.

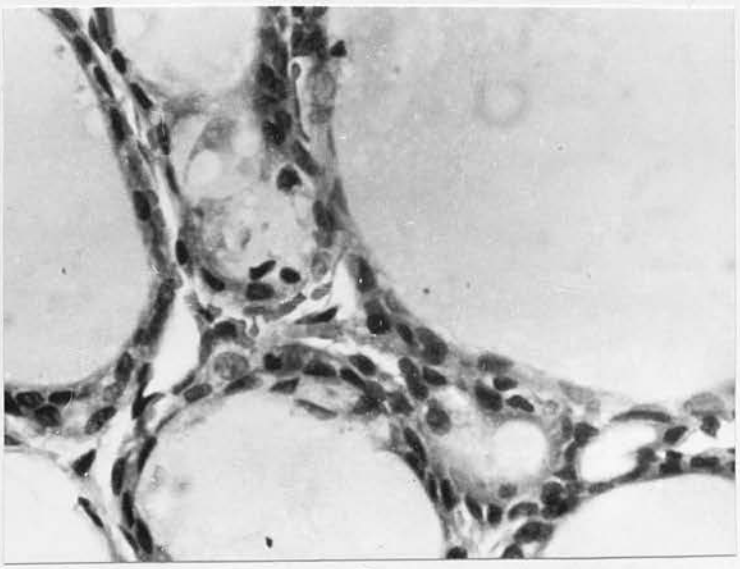

Fig. 62. High power of above rat thyroid gland, showing detail of height of epithelium.
H. & E. x 500.

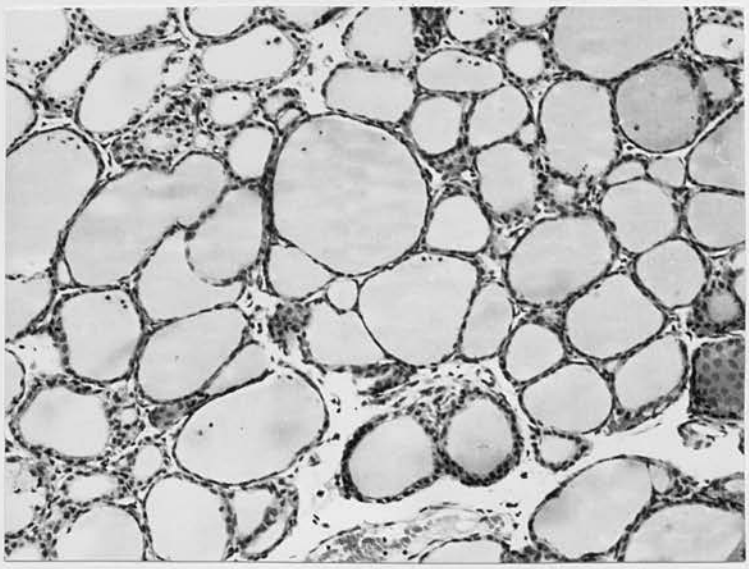

Fig. 63. Thyroid gland from a rat on moderate iodine diet and drinking water, with contained iodide supplement of 10 µg. /100 g. body wt./day in the water. Section shows slight decrease in height of epithelium compared to animal without iodide (Fig.61).

H. & E. x 125.

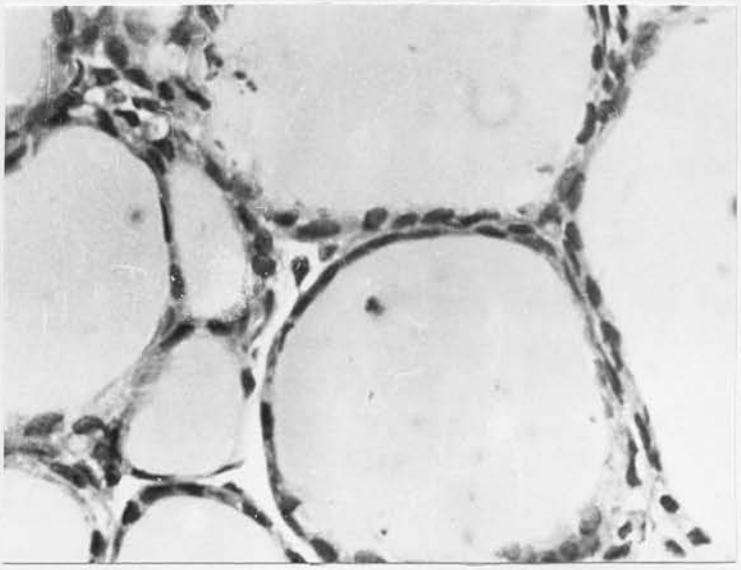

Fig. 64. High power of above rat thyroid gland, showing detail of epithelial height (cf. Fig. 62).

H. & E. x 500.

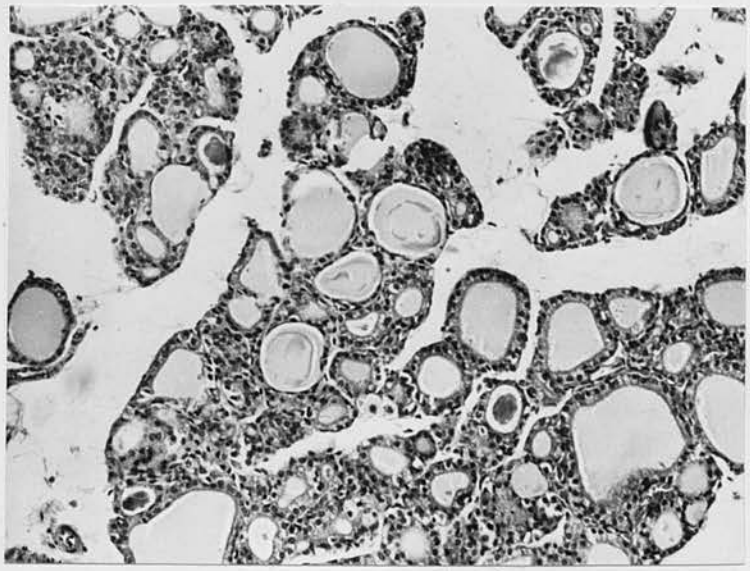

Fig. 65. Thyroid gland from a rat on moderate iodine diet and drinking reconstituted dried milk, prepared in April 1960, showing increased cellularity and height of epithelium lining of follicles, compared to Fig. 61. H. & E. x 125.

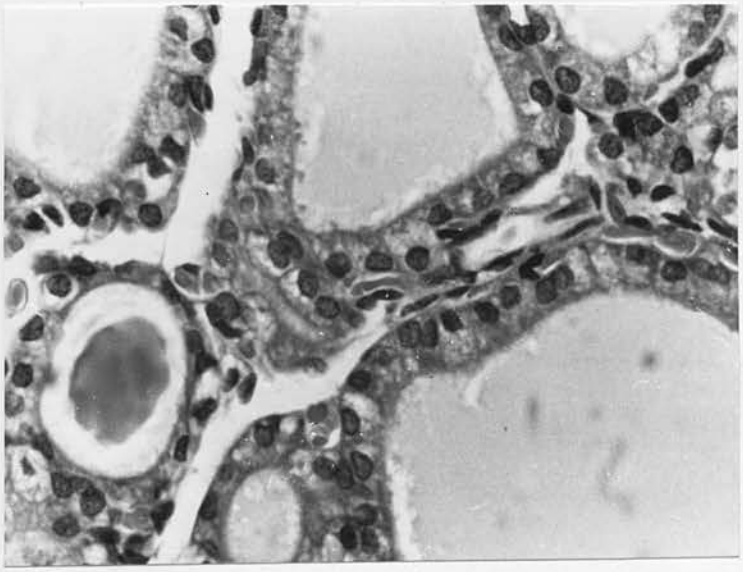

Fig. 66. High power of above rat thyroid gland, showing increase of height of epithelium, compared to Fig. 62. H. & E. x 500.

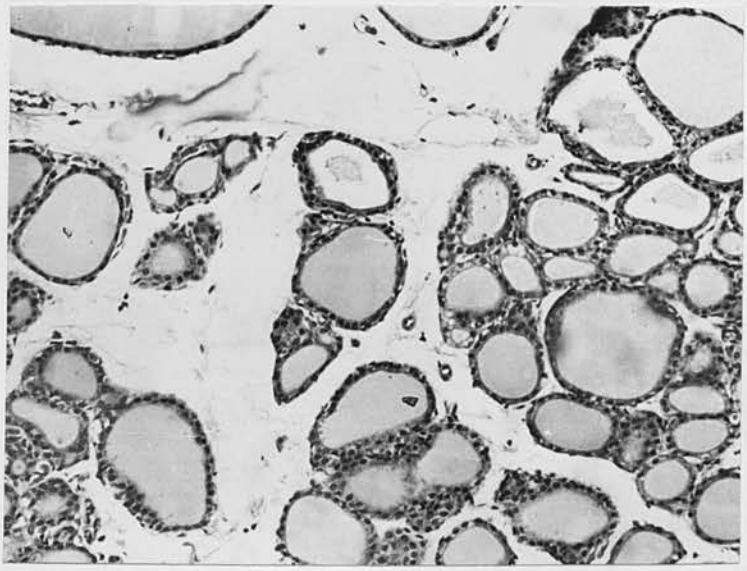

Fig. 67. Thyroid gland from a rat on a moderate iodine diet, and drinking reconstituted dried milk prepared in April 1960, with a contained iodide supplement of 10 μg. /100 g./day in the milk. Section shows considerable decrease in epithelial height, compared to milk without iodide (Fig. 65). H. & E. x 125.

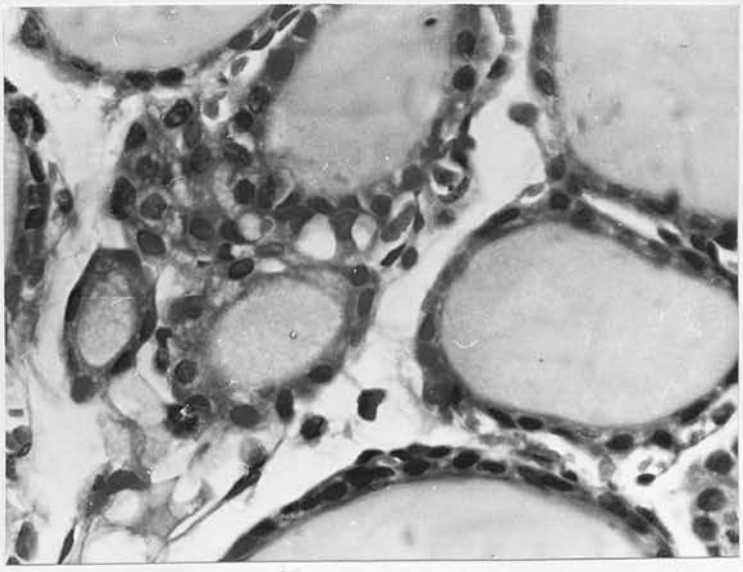

Fig. 68. High power of above rat thyroid gland, showing decrease of height of epithelium, compared to Fig. 66. H. & E. x 500.

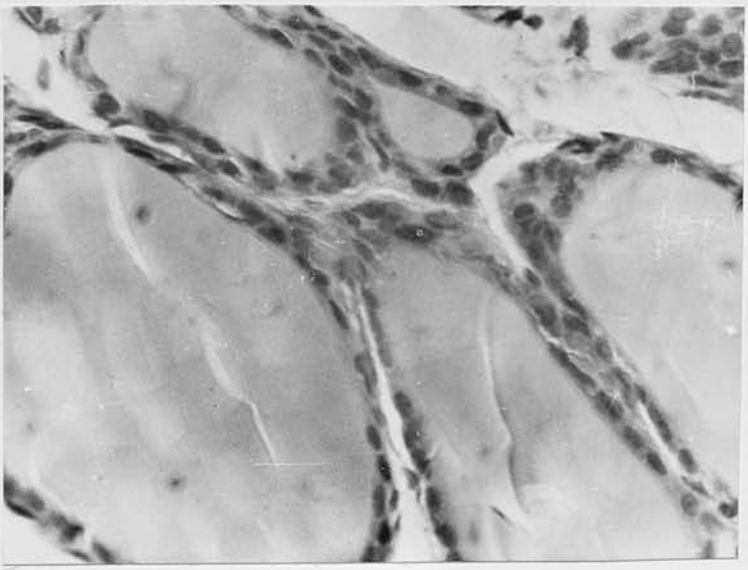

Fig. 69. Thyroid from a rat drinking water and showing appearances similar to Fig. 62. H. & E. x 500

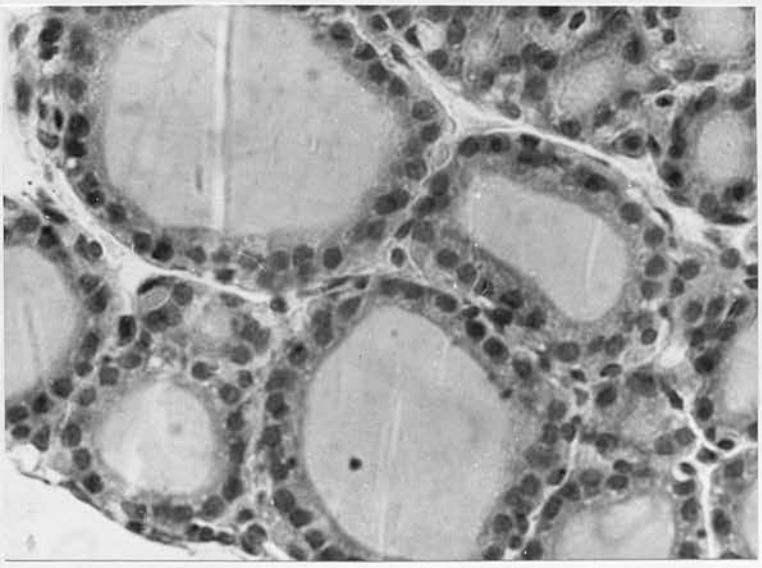

Fig. 70. Thyroid from a rat drinking reconstituted dried milk prepared in April 1960, showing hyperplasia compared to above. H. & E. x 500.

either dried milk alone or with milk and iodide. The increase of uptake found with goitrogenic activity in fresh milk during March - May, 1959 was therefore not demonstrated in the dried milk experiments. A further chronic feeding experiment using dried milk prepared in September 1960 had no goitrogenic activity, and this also agreed with the acute experiment on this batch of dried milk.

The thyroid hyperplasia produced by dried milk of April 1960 for 54 dy. is shown in Figs. 61 - 70, along with the effect of added iodide and sections from control rats. There is slight but significant increase in follicular cell height in the animals taking milk alone and this is reduced by iodide supplementation. These appearances will be compared with those produced by varying amounts of known anti-thyroid substances in Chapter 8.

Cause of antithyroid activity in milk

Iodide can produce depression of ^{131}I uptake, when given in sufficient amounts. The iodide concentrations of all the samples of fresh and dried milk, which were used in the acute experiments, have been measured, and all were considerably lower than 100 µg./litre. Thus in these experiments the animals did not receive more than 1 µg. in 10 ml. of fresh milk or 2 µg. in 3 g. of dried milk. The effect of iodide on 4 hr. ^{131}I uptake is shown in Table 27.

Table 27. Effect of iodide and calcium on ^{131}I uptake of thyroid in rats

Treatment	Mean depression of 4 hr. ^{131}I content of thyroid as % of control group	No. of rats treated	No. of controls
Iodide 0·1 µg.	8 N.S.	10	10
Iodide 1 µg.	4 N.S.	8	8
Iodide 10 µg.	11 N.S.	8	8
Iodide 50 µg.	52 **	8	8
Iodide 500 µg.	95 **	8	8
Calcium 7·5 mg.	14 N.S.	10	10

Iodide was given orally as potassium iodide. Calcium was given orally as calcium nitrate. Both were given 1 hr. before ^{131}I.

Clearly depression was not produced unless more than 10 µg. of iodide was given, and the iodide content of milk given cannot be the explanation of depressed 4 hr. uptake by milk. A similar experiment with calcium demonstrates that an amount of calcium equal to that present in the milk, has no effect on 4 hr. uptake of ^{131}I, and is also shown in Table 27.

Calcium has been eliminated as the cause of the goitrogenic activity of the milk, as shown in Table 28 . Rats fed on a low iodine diet (50 µg/kg) were also given calcium nitrate in their drinking water to reproduce the calcium intake from milk. There was no significant change in thyroid weight after 3 mths, but a significant depression of 24 hr. uptake.

Studies on thiocyanate content of milk were also carried out. Milk was collected weekly from a single supplier in Sheffield and the thiocyanate concentration measured by the method of Bowler (43). The concentrations from October 1959 to October 1960 are shown in Fig. 71. The thiocyanate concentration varied between 5 and 10 mg./litre and appeared to be lowest in April. As shown in Table 28 , there was no goitrogenic activity using the upper level of thiocyanate found in milk. The results of uptake are again difficult to explain, as there was depression with thiocyanate and increase with

Table 28 Effect of calcium and thiocyanate on thyroid weight and ^{131}I uptake in rats

Treatment	Duration of treatment (Days)	Mean increase in thyroid weight /mg./ 100 g. as % of control group	Mean 24 hr. ^{131}I content of thyroid as % of control group	No. of rats treated	No. of controls
Calcium nitrate	90	- 5 N.S.	59 **	9	8
Calcium nitrate + iodide	90	17 N.S.	77 *	9	8
Potassium thiocyanate	90	-12 N.S.	45 **	12	12
Potassium thiocyanate + iodide	90	0	168 *	12	12

All rats were on a low iodine diet - 50 µg. I./kg.
Calcium and thiocyanate were given in drinking water as calcium nitrate 296 mg./ litre and potassium thiocyanate 10 mg./litre.
Iodine supplement was 15 µg./rat/day, given as potassium iodide in drinking water.

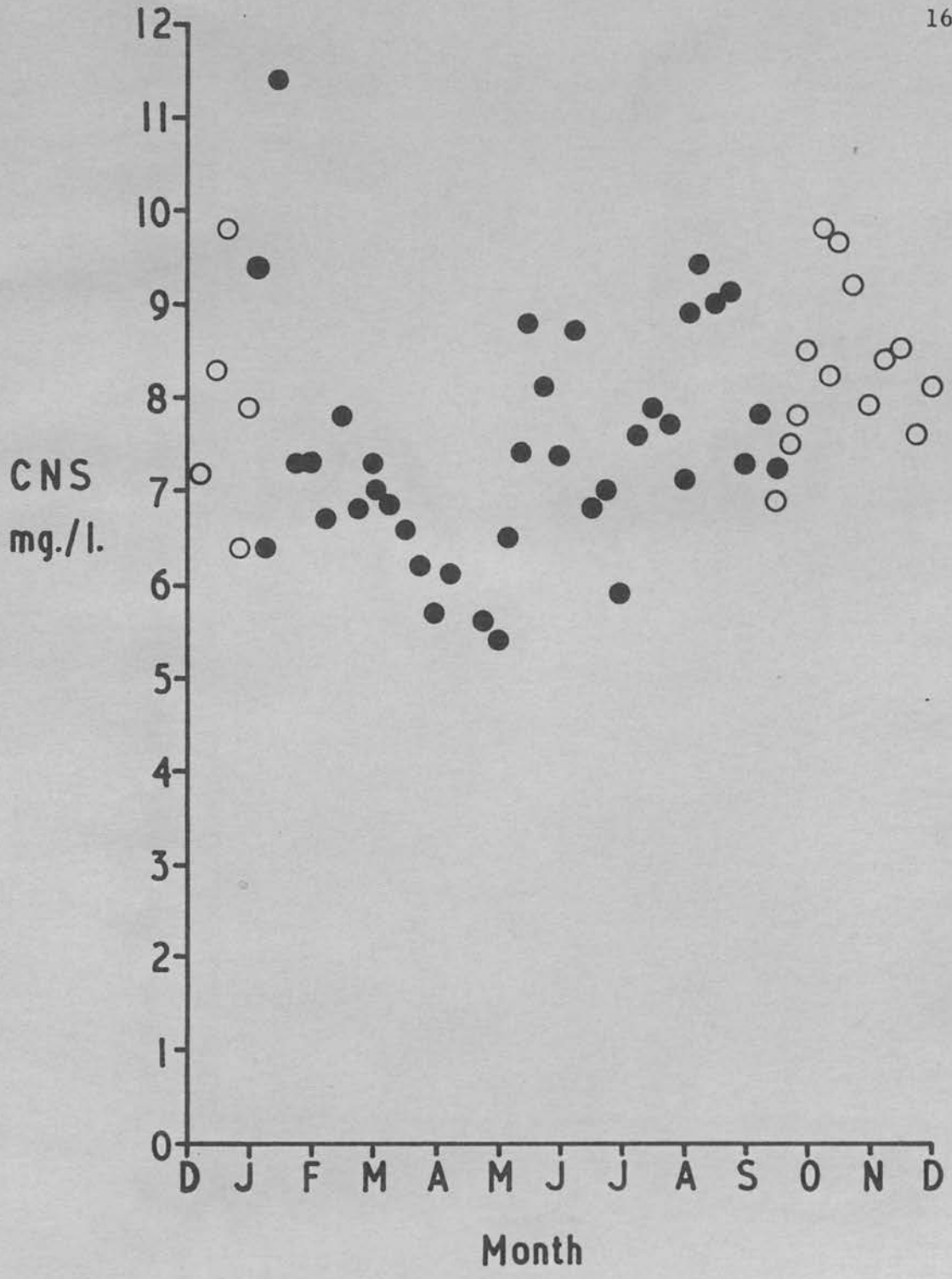

Fig. 71. Mean thiocyanate content of milk from four regions supplying milk to Sheffield during 1959 (open circles) and 1960 (closed circles).

thiocyanate and added iodide. A demonstration that such thiocyanate concentrations in milk are insufficient to discharge trapped iodide from the human thyroid is shown in Fig. 72 . A female patient, previously hyperthyroid and controlled by carbimazole therapy, was given 30 mg. of carbimazole to block organic binding of iodide, and 2 hr. later drank 500 ml. of fresh milk. The slow fall in thyroid uptake was not due to the milk, as it occurred the following week, when water was taken instead of milk. On both occasions the trapped iodide was completely discharged by potassium perchlorate at the end of the experiment. The slow fall in uptake is due to falling plasma ^{131}I concentration, as a result of renal excretion of ^{131}I.

The final experiment demonstrates that the antithyroid activity of milk is similar in type to the antithyroid drugs such as carbimazole, which block organic binding of iodine in the thyroid. Their presence can be detected by observing a discharge of iodide from the thyroid by either thiocyanate or perchlorate. The results are shown in Table 29 . The depression of 4 hr. uptake is significantly lower after dried milk followed by thiocyanate compared to dried milk alone. This strongly suggests that the thiocyanate has discharged trapped iodide, which has not been organically bound due to some factor in the milk.

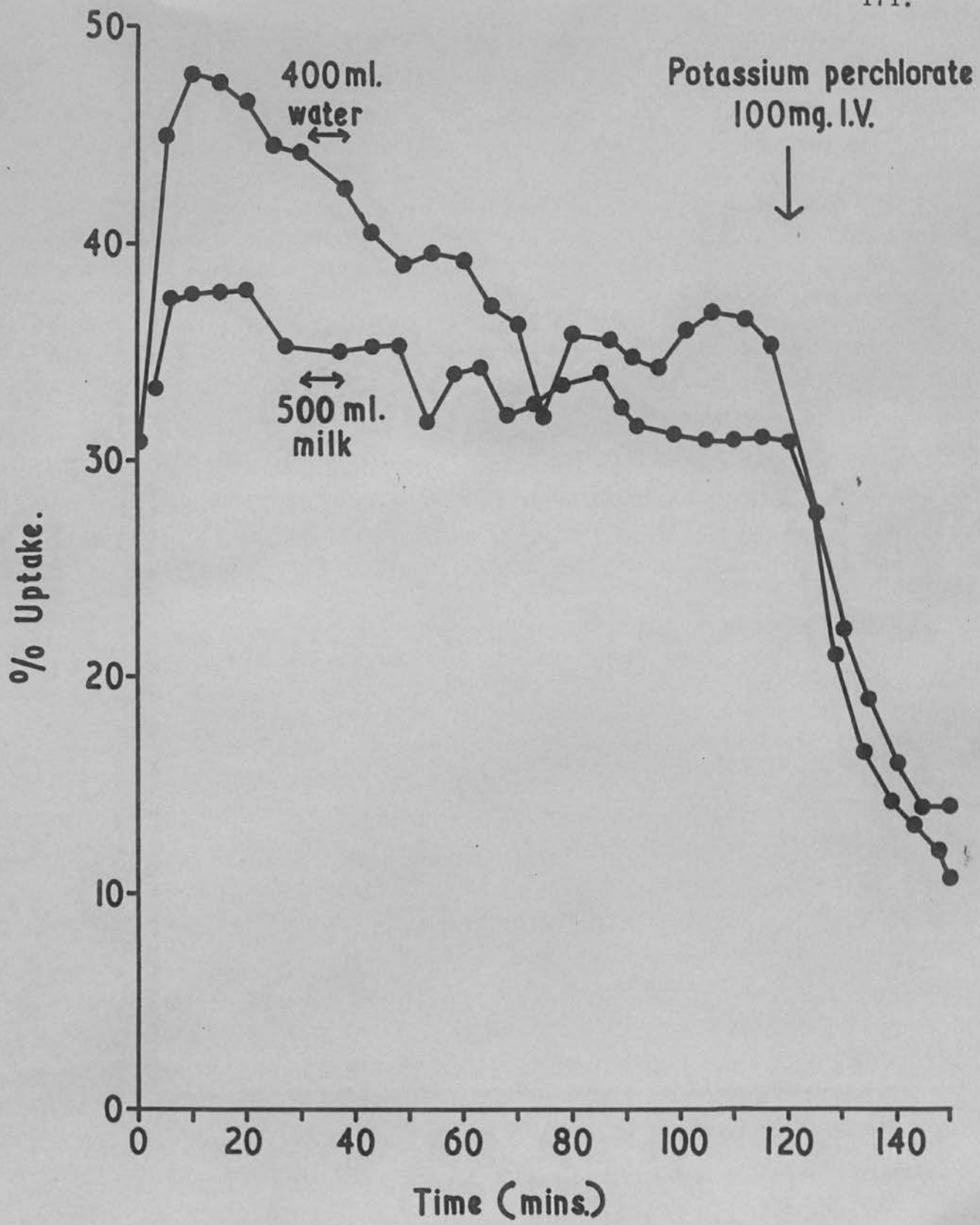

Fig. 72. Lack of effect of milk in discharging non-organically bound ^{131}I from human thyroid gland. Female patient E.L. on maintenance therapy with carbimazole for hyperthyroidism was given 30 mg. of carbimazole 1 hr. before ^{131}I was given intravenously. Either milk or water was drunk 30 min. after ^{131}I. A striking discharge of ^{131}I was produced by 100 mg. of potassium perchlorate given 2 hr. after ^{131}I.

Table 29. Effect of potassium thiocyanate on the depression of ^{131}I uptake produced by dried milk, prepared in April, 1960

Treatment	Mean 4 hr. ^{131}I content of thyroid (% dose + s.e.	Mean depression of ^{131}I content of thyroid as % of control group	No. of rats
Dried milk (April, 1960) 2·5 g.	4·2 + 0·61	43**	10
Dried milk + potassium thiocyanate	2·8 + 0·28	56**	10
Water	7·3 + 0·85	-	10
Water + potassium thiocyanate	6·4 + 0·79	-	10

Dried milk was given as two oral doses of 1·25 g. separated by an interval of 1 hr. ^{131}I was given 1 hr. after 2nd dose. 1 mg. potassium thiocyanate was given i.p., 2 hrs. after ^{131}I and rats were killed 1 hr. later.

Fresh milk from Sheffield and Ormiston compared by effect on radioiodine uptake by rat thyroid

While the present thesis was being prepared, fresh milk has been obtained monthly from Ormiston and tested by effect on 4 hr. uptake of ^{131}I as previously described. Milk from the usual distributor in Sheffield was tested at the same time for comparison. The results between September 1960 and March 1961 are shown in Table 30 . No antithyroid activity was demonstrable from either place until March, which confirms the findings with Sheffield milk of the previous year. Significant depression of uptake occurred in March and was greater in Sheffield milk than that from Ormiston. Unfortunately dried milk is not manufactured around Edinburgh and therefore milk from this region has not been tested for goitrogenic activity. It has not proved feasible to test this with liquid milk, due to the difficulties of sending liquid milk from Ormiston to Sheffield daily for some weeks.

Table 30. Antithyroid activity in rats of fresh liquid milk from Sheffield and Ormiston, as tested by two oral doses of 5 ml., separated by 1 hr.

Date of Test	Mean depression of 4 hr. ^{131}I content of thyroid as % of control group	
	Sheffield	Ormiston
30. 9. 60.	4 N.S.	-3 N.S.
28.10. 60.	5 N.S.	9 N.S.
25.11. 60.	1 N.S.	-6 N.S.
16.12. 60.	7 N.S.	-8 N.S.
20. 1. 61.	14 N.S.	-1 N.S.
3. 3. 61.	45**	24*
24. 3. 61.	28*	11 N.S.

^{131}I was given 1 hr. after 2nd dose of milk and rats killed after further 4 hr.

in thyroid weight calculated on the basis of body weight. Axelrad, Leblond and Isler (23) presented very convincing evidence that calcium had no effect on the thyroid weight in mice. This is confirmed by the present work.

Considerable evidence that thiocyanate is found in certain plants has been given by Michajlovskij and Langer (244, 245). These workers (189, 190) have shown rises in blood thiocyanate concentration in guinea-pigs after ingestion of Brassica, and depression of ^{131}I uptake. In New Zealand, Flux et al. (108) and Butler et al. (51) have shown that the high cyanide content of white clover is associated with high thiocyanate levels in sheep eating the clover and depression of ^{131}I uptake. Wald et al. (364) reported in 1939 that the blood level of thiocyanate in man was 8 - 10 mg./100 ml. in patients who developed goitre when treated with thiocyanate for hypertension. This level required doses of 1·0 g. of potassium thiocyanate daily, and it is very unlikely that such levels could be produced except by direct ingestion of very large amounts of Brassica e.g. 10 kg. However there is little doubt that it may be an important factor in the production of goitre in animals (Clements, 70). It seems certain from the evidence of thiocyanate concentrations found in milk in Sheffield that thiocyanate does not account for the goitrogenic property of milk.

The evidence that substances of the type which cause block of organic binding of iodine, occur in nature is convincing. Three derivatives of 2-thio-oxazolide have been isolated; 5:5 dimethyl-2-thio-oxazolidone from Conringia orientalis by Hopkins (154) and Kjaer et al., (179); 1-5-vinyl-2-thiooxazolidone from many species of Brassica, both in seeds and leaves, by Astwood et al.(18), Virtanen et al. (360) and Altamura et al. (6); 5-phenyl-2-thiooxazolidone from Barbarea vulgaris (winter-cress) and Reseda luteola by Kjaer and Gmelin (177, 178). The first two have been shown to have goitrogenic properties in animals by Astwood et al.(18). They found that 1-5-vinyl-2-thiooxazolidone was equal in activity to thiouracil in man, but only one-fifth as active as thiouracil in the rat. Greer (128) showed that 1-5-vinyl-thiooxazolidone (goitrin) was released by enzymatic hydrolysis from an inactive precursor called progoitrin. This hydrolysis was prevented

by cooking Brassica, but Greer and Deeney (131) have since shown that progoitrin has similar anti-thyroid activity to goitrin when given to man and is probably hydrolysed to goitrin in the absence of the vegetable enzyme. Direct ingestion of above plants apart from Brassica by man is unlikely, but many are fed to cows or occur as weeds in pasturage. Thus the possibility of their presence in cow's milk must be considered. Virtanen et. al.(360, 361) and Kreula and Kiesvaara (182) have demonstrated the presence of 1-5-vinyl-2-thiooxazolidone in milk of cows eating various species of Brassica, and shown that up to 0·07% of the amount ingested is excreted in milk. The highest concentration found was 100 µg./litre of milk. Attempts have been made by the author to measure this compound in the milk from Sheffield.

by the method of Kreula and Kiesvaara (182), but the method has been found to be technically difficult and unreliable. Certainly this upper level found by these authors (182) would not produce goitre in rats, according to its activity given by Astwood et al. (18) and the results given in Chapter 7.

Clements and Wishart (71) studied the depression of accumulation gradient of ^{131}I in human volunteers after drinking milk from cows feeding on various pastures and chou moellier (kale). A variable effect was found, but significant depression occurred with kale milk, milk from pastures contaminated with wild turnip weed, and milk from pastures with cruciferous weeks. Greene, Farran and Glascock (122) found significant depression of ^{131}I uptake in human volunteers, when milk from pastures lightly contaminated with cruciferous weeds was drunk for 4 weeks, and less effect with milk from cows fed on kale. The striking seasonal incidence of antithyroid activity in rats of milk in Sheffield would also suggest that kale is not implicated. By March cows begin grazing on pasture, and the present results suggest that growth of pasture in April is associated with considerable antithyroid activity. Cows are fed on kale during the winter, when the antithyroid activity of milk in Sheffield is low or absent. The seasonal incidence of goitrogenic activity in milk from Sheffield

is striking in relation to the findings of Gibson, Howeler and Clements in Tasmania (116). They have shown a seasonal increase in thyroid enlargement in school children, and this occurs each spring, when there is rapid growth of pasture and weeds. A goitrogenic substance in milk has been postulated by them to account for this effect. Peltola and Krusius (265) reported that milk from goitrous regions of Finland produced thyroid enlargement in rats and that milk from regions without goitre had no effect. It is interesting that ^{131}I uptakes were increased after prolonged administration of milk, and were 2½ times the uptakes of control rats after 1 yr. The present results confirm that depression of uptake does not occur when goitre results from milk administration and that a significant increase in uptake may occur. The significance of these observations will be discussed more fully in Chapter 7 where experiments producing high uptakes and goitre in rats by carbimazole are described.

It seems likely that the goitrogenic properties of milk are due to some other substance than 1-5-vinyl-2-thio-oxazolidone from the results of Virtanen et al. (360)Clements (70) states that γ-methylsulphonyl prophyliso-thiocyanate has been isolated from wild turnip weed, but it has apparently not yet been isolated from the milk which he found contained

goitrogens in Tasmania. It is not known whether this substance has properties like thiocyanate or blocks organic binding of iodine.

The effect of added iodide on the activity of natural goitrogens is variable. In Sheffield a partial inhibition of goitrogenic activity of milk was found. Care (57), Wright (389,390, 391) Wright and Sinclair (392,393) and Sinclair and Andrews (313,314) all noted that goitre in lambs due to ingestion of linseed meal and kale was prevented by iodide supplements. These workers suggest that thiocyanate is the goitrogen concerned because its goitrogenic activity is abolished by iodine. Astwood (11) showed that iodine completely prevented goitre production by thiocyanate. The above work on goitre in lambs also suggested the presence of inhibitors of organic binding of iodine, as do the present results. The influence of iodine on the action of organic iodine inhibitors such as thiouracil is complicated. McKenzie (218) has demonstrated that small amounts of iodine can partially inhibit the goitrogenic activity of thiouracil in rats, but this effect is not increased by adding more iodine. These results were confirmed by McGinty (209), Ferguson and Sellers (101) and by Follis (109,110). The enlargement in rat thyroid glands due to milk in Sheffield was hyperplastic in type, but McKenzie (218) and Follis (109,110) have been able to produce goitres

filled with colloid in rats by appropriate regimes of thiouracil and iodine.

The significance of demonstrations of goitrogenic activity of milk in rats to the problem of production of simple goitre in man is difficult to assess. Antithyroid activity of substances in man are assayed by measuring changes in accumulation gradient of ^{131}I, not by the production of goitre. Evidence will be presented in Chapter 8 that results of ^{131}I depression in rats do not always correlate with ability to produce goitre. Further, antithyroid activities in man need not correspond to that in animals. Stanley and Astwood (327) showed that propylthiouracil was equal in activity to thiouracil in man, but Astwood et al. (17) found that a ratio of 10 in the rat. Similarly methimazole is 100 times as effective as thiouracil in man (327), but only twice as active in the rat (17). Clearly the goitrogenic activity of any substance in man can only be assessed by human experiments. There is also considerable evidence that antithyroid substances have greater effect on young animals (391, 392, 393), and therefore the significance of natural goitrogens may be greater in children and adolescents. One further difficulty is to correlate the findings in human simple goitre with the action of goitrogens, and in particular the increased uptake found in simple goitre. Results on uptakes resulting from administration of inhibitors

of organic binding of iodine will be presented in the following chapter.

Summary

1. Fresh and dried milk produces depression of ^{131}I uptake in rats and accumulation gradient of ^{131}I in man. This effect is seasonal and greatest in April.

2. Such milk also produces thyroid enlargement and hyperplasia in rats, when fed adlibitum for some weeks. This effect is also seasonal and is not accompanied by depression of ^{131}I uptake. Iodine partially inhibits this goitrogenic action of milk.

3. These effects are not due to the iodine, calcium, or thiocyanate content of milk.

4. Evidence is given that organic binding of iodine is inhibited , when a single large dose of milk is given to rats.

5. The seasonal antithyroid activity of liquid milk from Sheffield has been confirmed for 1961. Milk from Ormiston also shows this effect but the degree of activity is significantly less than that from Sheffield.

7

ASPECTS OF IODINE METABOLISM IN EXPERIMENTAL GOITRE

The depression of ^{131}I uptake in rats by single large doses of milk, and the lack of depression and occasional increase of uptake at the end of prolonged administration of milk is apparently paradoxical. A conceivable explanation is the different concentration of goitrogenic material acting on the thyroid during the two experiments. A rapid increase in blood goitrogen concentration followed by rapid decrease will occur after a single dose of milk, whereas there will be a lower and more steady concentration during the chronic feeding experiment. The evidence given in Chapter 6 suggests that the acute depression of uptake by milk is associated with impaired organic binding of iodine in the thyroid. There is considerable evidence that antithyroid substances with this action produce depression of ^{131}I uptake, both when given as a single dose and by chronic administration (327, 304, 305, 174). However, there is little work reported on the effect of small doses of such substances given in the diet for long periods. It has been assumed that the sole mechanism of action of such compounds is inhibition of organic binding of iodine, and therefore no goitrogenic effect would be expected unless uptake of iodine

were depressed. Recently Slingerland et al.(318) reported occasional high uptakes of ^{131}I following administration of small amounts of propyl-thiouracil for 10 days, and suggestive evidence that such small doses blocked the conversion of moniodotyrosine to diiodotyrosine and thyronines without inhibition of organic binding of iodine to tyrosine. It seemed relevant to confirm that high uptake of ^{131}I could be produced by anti-thyroid substances, as high uptake is a striking feature of simple goitre in man.

Effect of carbimazole on thyroid weight and uptake

Young rats (60 - 80 g.) were given various concentrations of carbimazole in their drinking water. Carbimazole, unlike the thiouracils, has no taste and its presence in water does not affect the intake of water by rats. The diet was commercial cube diet giving a moderate iodine intake of 2-2·5 µg. iodine per day. The effect of three different dose levels of carbimazole on thyroid weight, uptake, and total thyroid iodine are shown in Table 31 . Increase in thyroid weight was produced by 100 µg. and 60 µg. per day, but no significant effect on 24 hr. uptake after administration for 40 days. However, it is noteworthy that goitre was produced without decrease in uptake. Total thyroid iodine was reduced with all doses. When the experiment was prolonged to 140 days, significant thyroid enlargement was present with 100 µg./day and

Table 31. Effect of small doses of carbimazole on thyroid weight, ^{131}I uptake and total iodine in rats

Treatment	Duration (days)	Mean increase in thyroid weight/mg./100 g. as % of control group	Mean 24 hr. ^{131}I content of thyroid as % of control group	Mean total thyroid iodine (µg.) as % of control group	No. of rats treated	No. of controls
Carbimazole (100µg./dy.)	40	136 *	93 N.S.	48 **	14	12
Carbimazole (60 µg./dy.)	40	119 *	84 N.S.	57 **	12	12
Carbimazole (20 µg./dy.)	40	110 N.S.	88 N.S.	74 *	12	12
Carbimazole (100µg./dy.)	140	126 *	172 **	36 **	6	6
Carbimazole (60 µg./dy.)	140	105 N.S.	120 *	39 **	6	6
Carbimazole (20 µg./dy.)	140	106 N.S.	94 N.S.	42 **	6	6

The significance of differences between control and treated animals was calculated using Student's t test and indicated thus

** $P < 0.01$; * $0.05 > P > 0.01$; N.S. $P > 0.05$.

24 hr. uptake significantly increased with both 100 µg./day and 60 µg./day regimes. Total thyroid iodine was lower with all three doses than on the 40 day experiment.

The effect of added iodide on these effects of carbimazole are of importance, and further experiments were carried out. The results are shown in Table 32 . Carbimazole (100 µg./day) given for 150 days again produced an increase in thyroid weight and increased 24 hr. uptake of ^{131}I. When iodide was given as potassium iodide in the drinking water along with the same amount of carbimazole, the uptake was not different from control animals given only iodide, and a partial reduction of the thyroid weight increase occurred. Other rats, which had been treated with either carbimazole and iodide, or iodide alone, were given potassium thiocyanate 2 hrs. before the end of the 24 hr. period for ^{131}I collection at the end of the experiment. No significant difference between these two groups was found, which is evidence that this dose of carbimazole had not produced any impairment of organic binding of ^{131}I, as a discharge of ^{131}I would be expected if such impairment were present.

Table 32. Effect of added iodide on the increased uptake of ^{131}I from carbimazole, and of thiocyanate given at end of the experiment

Treatment	Duration (days)	Mean thyroid weight (mg. ± s.e.)	Mean 24 hr. ^{131}I content of thyroid (% dose ± s.e.)	No. of rats
Carbimazole (100µg./dy.)	150	31·9 ± 1·31	11·8 ± 0·92	12
Carbimazole (100µg./dy.) + Iodide (3 µg./dy.)	150	26·8 ± 1·06	7·5 ± 0·66	12
Iodide (3 µg./dy.)	150	24·9 ± 0·92	8·1 ± 0·23	12
Carbimazole + Iodide + Thiocyanate*	150	23·6 ± 0·86	7·4 ± 0·46	6
Iodide + Thiocyanate*	150	23·8 ± 0·88	8·3 ± 0·31	6

* Potassium thiocyanate 10 mg. given intraperitoneally 22 hrs. after ^{131}I.

Effect of carbimazole on distribution of intra-thyroidal iodine

This work is still in a preliminary stage, but the results are shown in Table 33 . Rats given carbimazole at a dose of 500 µg./day for 10 days show the conventional effects of organic inhibition of iodine. These are increased thyroid weight, decreased 24 hr. thyroid uptake of ^{131}I, and decreased concentration of thyroid iodine. The ratio of radio monoiodotyrosine to radio diiodotyrosine (MIT/DIT) as found by chromatography of aliquots of the digested thyroid gland is considerably increased above the control value, and almost all the radioactivity is present as radio monoiodotyrosine. With 100 µg./day carbimazole for 10 days significant differences are observed from the higher dose level. Thyroid enlargement is less, but 24 hr. uptake is higher than the control value. Ratio of $MIT_{/DIT}$ is still three times higher than the control. Though no significant effect on thyroid weight or uptake is found with 60 µg./day or 20 µg./day for 10 days, total thyroid iodine is still significantly lowered, and ratio of $MIT_{/DIT}$ higher than normal. These results suggest an effect of carbimazole on stages of hormone synthesis after the formation of monoiodotyrosine.

Table 33. Effect of carbimazole for 10 days on thyroid weight, ^{131}I uptake, total iodine and ratio of radiomonoiodotyrosine to radiodiiodotyrosine

Treatment	Mean thyroid weight (mg. ± s.e.)	Mean 24 hr. ^{131}I content of thyroid (% dose ± s.e.)	Mean total thyroid iodine (µg.) ± s.e.	Mean MIT/DIT ratio ± s.e.	No. of rats
Water	17·8 ± 1·04	10·1 ± 1·21	15·2 ± 1·2	0·56 ± 0·08	6
Carbimazole (500 µg/dy.)	26·6 ± 1·23	6·0 ± 1·01	3·6 ± 0·76	12·20 ± 1·02	6
Carbimazole (100 µg./dy.)	23·9 ± 1·31	13·5 ± 1·45	7·7 ± 0·92	1·34 ± 0·65	6
Carbimazole (60µg./dy.)	18·6 ± 0·94	12·9 ± 1·31	9·4 ± 1·03	0·96 ± 0·14	6
Carbimazole (20µg./dy.)	17·2 ± 0·98	11·2 ± 1·06	11·9 ± 1·16	0·78 ± 0·09	6

Discussion

It is clear from the above results that uptake of ^{131}I may be increased when slight degrees of thyroid enlargement are produced by small doses of carbimazole. This effect may be explained by inhibition of hormone synthesis after the formation of monoiodotyrosine and by this stage being affected by amounts of carbimazole which are insufficient to prevent iodination of tyrosine. The early findings of Astwood and Bissell (16) in 1944, that total thyroid iodine was reduced by administration of thiouracil, and the evidence that remaining thyroid radioactivity was present as iodide (16, 209), strongly suggested that the action of thiouracil was to block organic binding of iodine to tyrosine. Other workers showed such an inhibition by thiouracil on thyroid slices *in vitro* (111, 112, 113, 172). Even in 1943, Dempsey and Astwood (87) showed that the goitrogenic action of thiouracil was prevented by thyroxine, but not by diiodotyrosine. This raised the possibility that thiouracil might also block thyroid hormone synthesis beyond iodination of tyrosine. However if this were so, an accumulation of iodine bound to tyrosine might be expected after thiouracil. Such evidence was not found by Franklin and Chaikoff (111, 112). Franklin *et al.*, (113), Keston *et al.* (172), or by Dempsey and Astwood (87). Later Pitt-Rivers (272) reviewed the possibility

that antithyroid compounds might inhibit the coupling of diiodotyrosine to tetraiodothyronine, and showed that such an inhibition could be demonstrated *in vivo* (271). Gross and Leblond (133), and later Halmi and Stuelke (140) reported that some ^{131}I was not dischargeable from the thyroid by perchlorate after treatment with propyl-thiouracil. Pitt-Rivers *et al.* (273) then showed that such radioactivity was present as monoiodotyrosine. The present work and the results of Slingerland *et al.* (318) confirm that there are increased amounts of moniodotyrosine in the thyroid after treatment with small amounts of carbimazole or propylthiouracil, and that the ratio of MIT/DIT is increased and concentration of iodine decreased, even when no change in thyroid weight is produced. As the stage of hormone synthesis after formation of monoiodotyrosine is apparently more sensitive to antithyroid compounds, it seems reasonable that increased uptakes of ^{131}I may occur due to increase in the rate of formation of MIT. That such increases may occur, but inconsistently is shown by the present results. Astwood (14) noted that patients with hyperthyroidism could be restored to a euthyroid state by antithyroid drugs, and goitre produced in euthyroid individuals by such drugs, and yet these patients could still have high uptakes of ^{131}I present as

bound iodine. The effect on inhibition of synthesis after MIT offers an explanation for these high uptakes, which were observed while the treatment was continued. Many workers have demonstrated a rebound increased uptake after cessation of antithyroid drug therapy (169, 370, 375), but this appeared more explicable compared to high uptakes while on therapy. The relevance of this demonstration of high uptakes to the aetiology of simple goitre is obvious and important, as is the demonstration that high uptakes, due to antithyroid compounds, can be prevented by added iodide. Pitt-Rivers et al. (274) and Dimitriadou et al.(89) reported that increased amounts of radiomonoiodotyrosine were found in human simple goitre in England and suggested that a defective iodinating mechanism accounted for this. Clearly small amounts of goitrogens will produce the same effect, and cannot be dismissed as contributing to the production of simple goitre because of such evidence. Thus both iodine deficiency (281) and antithyroid substances produce an increased ratio of MIT/DIT, and this is also found in specific types of inherited dyshormonogenesis (McGirr, 213). The importance of these causes in the production of simple goitre in man must be evaluated on the basis of other evidence.

Summary

1. Increase in thyroid weight and 24 hr. uptake of ^{131}I has been produced in rats by small amounts

(100 µg./day) of carbimazole.

2. The increased uptake of ^{131}I due to small amounts of carbimazole can be prevented by added iodide, and the increase in thyroid weight partially prevented.

3. The ratio of monoiodotyrosine to diiodotyrosine is increased by carbimazole, even when the amounts given are insufficient to produce thyroid enlargement.